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(54) **Title:** BIOPOLYMER THICKENER

(57) **Abstract:** A novel strain of *Lactococcus lactis* subspecies *cremoris* ("Ropy 352") has been identified and isolated. Ropy 352 produces a previously unknown exopolysaccharide (EPS 352) that when expressed in or added to milk, imparts highly desirable sensory characteristics to the milk, including making the milk very thick, with a very smooth mouth-feel, and slightly sweet with an obvious "chewable-bite".

BIOPOLYMER THICKENER

ACKNOWLEDGMENT OF GOVERNMENT SUPPORT

This invention was made in part with government support under The National Dairy Promotion and Research Board (i.e. Dairy Management Inc., DMI) and USDA/CSREES Special Research Grant. Accordingly the government has certain rights in this invention.

FIELD OF INVENTION

The field of the invention relates to biopolymers, enzymes that are contained within biopolymer synthesis pathways, nucleic acid sequences encoding such enzymes, and to organisms that make such biopolymers, wherein such biopolymers may be used to thicken liquids including liquid foods, as well as an additive to pharmaceuticals, beauty products, and coating agents.

BACKGROUND

Microbial polysaccharides are used for a broad variety of industrial applications including food production, chemical production (e.g., detergents, cosmetics, paints, pesticides, fertilizers, flocculants, film formers, lubricants and explosives), pharmaceutical production and waste treatment. In food production, microbial polysaccharides are commonly used as thickening, gelling and homogenizing agents. When added to a liquid, microbial biopolymers contribute to viscosity, emulsion stabilization, surface tension and adhesiveness. Thickening applications are particularly important in the production of solid and semi-solid food products including dairy and non-dairy foods such as yogurt, buttermilk, salad dressings, cheese, and ice-cream. Thickening of liquid foods is desirable because of consumer preference for such thickened foods, which have a characteristic texture and "mouth feel." Thickening of liquid drinks is also desirable for use with elderly people who frequently have problems swallowing low-viscosity liquids (e.g., milk and fruit juices) due to an impaired swallowing reflex. The addition of thickener to such drinks facilitates swallowing and reduces aspiration of liquid into the trachea.

Currently the only microbial polysaccharides used to any appreciable extent in industry are dextran, produced by *Leuconostoc mesenteroides*, xanthan gum, produced by *Xanthomonas campestris*, and gellan gum, produced by *Aureomonas elodea* ATCC31461 (Crescenzi, *Biotech. Prog.* **11**:251-259, 1995). Xanthan gum was approved by the U.S. Food and Drug Administration (FDA) for use in foods in 1969. Today it is used in many foods such as bakery fillings, canned foods, frozen foods, pourable dressings, sauces, gravies, processed cheeses, and juice drinks. Xanthan gum is also used in oil recovery, pharmaceuticals, beauty products, and coating agents.

Unfortunately, *Xanthomonas campestris* is a less than ideal source of polysaccharides for use in food production, since it is known to be pathogenic, and the biopolymer it produces has long been suspected of being pyrogenic (fever-inducing). Although xanthan gum is classified as "Generally Regarded as Safe" (GRAS) by the Food and Drug Administration (FDA), *Xanthomonas campestris* is not.

Lactic acid bacteria (LAB) are classified GRAS, and have been used for centuries in fermented dairy products such as yogurt, cheese, and sour-cream. A characteristic of some LAB in food production processes is their production of exopolysaccharides (EPS). EPS provide improved viscosity and mouth-feel while also preventing syneresis (separation) in fermented food products. Despite their ability to produce EPS, LAB are not generally used as sources of thickening agents (either within a milk-based culture or as a source of exogenous EPS) because the EPS-positive phenotype is readily lost (Dierkesen et al., *J. Dairy Sci.* **80**(8):1528-1536, 1997). The LAB strain described in this disclosure stably produces EPS when cultivated on appropriate media.

SUMMARY OF THE DISCLOSURE

A natural isolate of *Lactococcus lactis*, named "*Lactococcus lactis* subspecies *cremoris* Ropy 352," hereinafter referred to simply as "Ropy 352", has been isolated. This strain contains a plasmid (EPS plasmid) that encodes at least 13 active genes (Figure 3). The enzymes encoded by these genes allow the bacteria to produce a previously unknown exopolysaccharide ("EPS 352"). Hence, in addition

to providing EPS 352, the present invention also provides the nucleic acid sequences and the corresponding amino acid sequences of 13 of the open reading frames (ORFs; SEQ ID NO: 10) found on the EPS 352 plasmid.

EPS 352, when expressed in or added to milk or other liquids, imparts desirable sensory characteristics to the milk, including making the milk very thick, with a very smooth mouth-feel, and slightly sweet with an obvious "chewable-bite." Ropy 352 producing EPS, or EPS 352 alone may be added to any milk-based or non milk-based product, including any liquid food product, to produce these sensory characteristics. In the Ropy 352 strain, the biosynthesis of EPS 352 is controlled by genes carried outside the chromosome on a plasmid of about 32 kb ("EPS 352 plasmid"). Precedent predicts that the EPS 352 genes are linked in an operon like fashion. The EPS 352 plasmid has been isolated from the Ropy 352 organism, and the plasmid has been transformed into a plasmid free nonropy laboratory strain of *Lactococcus*, MG1363. (Gasson, *J. Bacteriol.* **154**:1-9, 1983.) The plasmid encoded EPS 352 genes are expressed in the transformed strain, producing a ropy EPS, which imparts desirable sensory characteristics (as detailed below) to milk-based media.

One aspect of the invention provides the isolated *Lactococcus lactis* subspecies *cremoris* Ropy 352 organism (Ropy 352) as deposited under the rules of the Budapest Treaty, USDA-ARS-NCAUR-NRRL deposit number NRRL B-30229. Ropy 352 can be added to liquids (e.g., solids, semi-solids and gels) to cause thickening. Such thickening is desirable for use in creating products such as food products, beauty care products, and pharmaceuticals. Additionally, the Ropy 352 organism can be used to produce food products by fermentation of a food substrate with a culture of the Ropy 352 organism. Accordingly, the invention also provides the products made through the addition of the Ropy 352 culture.

Another aspect of the invention provides the purified exopolysaccharide EPS 352. EPS 352 can be added to liquids to produce food products as well as other products such as pharmaceuticals. Examples of such liquids include, liquid food substrates, such as milk-based liquids, soy-based liquids, fruit juice, and whey-based liquids. Accordingly the invention also provides the products made through the addition of EPS 352.

Yet another aspect of the invention provides the plasmid (contained in the deposited bacterial strain NRRL B-30229) that contains the open reading frames that encode the enzymes necessary for the production of EPS 352. This plasmid is approximately 32 kb in size. The identification of the plasmid allows for the production of EPS 352 by transgenic organisms that have been transformed with the EPS 352 plasmid. Furthermore, these transgenic organisms can be added to liquids to generate food products.

Another aspect of the invention provides methods of using the individual enzymes encoded by the EPS 352 plasmid for the production of modified exopolysaccharides. Used in these methods the enzymes derived from the nucleic acid sequence of the EPS 352 plasmid can be combined with other genes that code for exopolysaccharide biosynthetic pathways enzymes such that the exopolysaccharide produced is distinct from that of the disclosed EPS 352. Furthermore, these methods can be practiced *in vitro* or *in vivo*. (Stingele et al., *Mol. Microbiol.* **32**(6):1287-1295, 1999; Kranenburg et al., *J. Bacteriol.* **181**(11):6347-6453, 1999; Stingele et al., *J. Bacteriol.* **181**(20):6354-6360, 1999; and Klerrebezem et al., *Antonie van Leewenhoek* **76**:357-365, 1999).

Another aspect of the invention provides methods of using EPS 352 in various pharmaceutical formulations. Used in this context EPS 352 can be incorporated dry into pill formulations or into liquids to increase the viscosity of the formulation and facilitate delivery of the active ingredients.

Another aspect of the invention provides methods of using EPS 352 in various beauty products, such as hair shampoos, hair bleaching compositions, hair conditioners, hair gels and mousse, skin creams, nail varnishes, facial foundation, skin tanning gels, hair removers, shaving creams and in pill coatings, children's products (i.e., crayons, non-toxic glues), in addition to various industrial processes. (Hilger et al., *J. Environ. Eng.* **125**(12):1113, 1999 and Shah et al., *Appl. Biochem. Biotech.* **82**(2):81, 1999.)

SEQUENCE LISTING

The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and three-

letter code for amino acids. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood to be included by any reference to the displayed strand.

SEQ ID NO: 1 shows the nucleic acid sequence of a portion of the EPS 352 plasmid.

SEQ ID NO: 2 shows the amino acid sequence of the enzyme designated "R" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

SEQ ID NO: 3 shows the amino acid sequence of the enzyme designated "X" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

SEQ ID NO: 4 shows the amino acid sequence of the enzyme designated "A" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

SEQ ID NO: 5 shows the amino acid sequence of the enzyme designated "B" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

SEQ ID NO: 6 shows the amino acid sequence of the enzyme designated "C" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

SEQ ID NO: 7 shows the amino acid sequence of the enzyme designated "D" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

SEQ ID NO: 8 shows the amino acid sequence of the enzyme designated "E" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

SEQ ID NO: 9 shows the amino acid sequence of the enzyme designated "O" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

SEQ ID NO: 10 shows the amino acid sequence of the enzyme designated "P" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

SEQ ID NO: 11 shows the amino acid sequence of the enzyme designated "F" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

SEQ ID NO: 12 shows the nucleic acid sequence encoding Eps "M" and Eps "N."

SEQ ID NO: 13 shows the amino acid sequence of the enzyme designated "N" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 12.

SEQ ID NO: 14 shows the amino acid sequence of the enzyme designated "M" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 12.

SEQ ID NO: 15 shows the nucleic acid sequence encoding the enzyme designated "U."

SEQ ID NO: 16 shows the amino acid sequence of Eps "U," which is encoded by SEQ ID NO: 15.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 describes the degree of phosphate protonation. As sodium hydroxide is added to the polysaccharide solution, there is only one inflection in the titration profiles, indicating that the phosphate group in the EPS 352 is in the form of a phosphodiester linkage rather than as the monoester, which would have shown 2 inflection points.

Figure 2 shows double stranded sequence data from the EPS 352 plasmid and the corresponding amino acid sequences named EpsM and EpsN. The insertion site of the IS*S1* element is indicated in EspN and which confers a non-ropy phenotype in Ropy 352, thus linking these two open reading frames to EPS 352 expression.

Figure 3 shows the alignments of the ORF designated "N" in Figure 4 and the ORF designated "M" in Figure 4 to each other as well as to an enzyme (EpsG) involved in eps biosynthesis in *Lactococcus lactis* NIZOB40. The overall identity between ORF "M" and EpsG is 24% and between ORF "N" and EpsG is 25%.

Figure 4 is a diagram of the organization of the genes on the EPS 352 plasmid. The large arrows with letters inside represent genes and their orientation. The square with the letter X is a non-functional gene as it is missing its beginning (5' prime sequence). Eps ORFs are designated M, N, O, and P. The site of the ISS1 insertion, which disrupted EPS 352 production, is indicated by an downward pointing arrow that points to a position in Eps N.

Figure 5 shows the DNA and amino acid sequence of the entire EPS operon from upstream of the promoter to downstream of the terminator. This sequence is

6850 bp in length. The starts of the open reading frames are labeled with the gene name (corresponding to Figure 4) printed in the right margin.

Figure 6 shows the nucleic acid sequence of Eps U. The start and stop codons are underlined.

DETAILED DESCRIPTION

DEFINITIONS and ABBREVIATIONS

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes VII*, Oxford University Press, 1999 (ISBN 0-19-879276-X); Kendrew et al. (eds.), *The Encyclopedia of Molecular Biology* Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

W/V means weight per unit volume.

kDa means kilodaltons.

MWCO means molecular weight cutoff

TCA means trichloroacetic acid.

Mol % means molar percent

mPA-s means millipascals

n.d. means none detected.

***Lactococcus lactis* subspecies *cremoris* Ropy 352 ("Ropy 352")** is the organism deposited under the Budapest Treaty as USDA-ARS-NCAUR-NRRL deposit number NRRL B-30229. Ropy 352 has the characteristic property of producing the exopolysaccharide EPS 352 under suitable growth conditions, e.g., streaked onto whey agar or defined lactococcal medium containing glucose agar plates and incubated at 30°C.

EPS 352 is an exopolysaccharide that is produced by Ropy 352 and that has the following characteristics:

Composition:	Glucose:	range of 54% to 58%
	Galactose:	range of 42% to 46%
Charged:	Yes	
Molecular weight:		range of 800,000 to 8,000,000

(average of 1,600,000)

Phosphorous: Present in backbone or sidechain

Structure: Endpoints: galactose; Branchpoints: glucose

Several gene products are required for EPS 352 biosynthesis. The EPS biosynthetic genes are located extrachromasomally on the EPS 352 plasmid. Precedent indicates that these genes are organized in an operon like fashion.

EPS 352 plasmid is an extrachromosomal plasmid of approximately 32 kb in size that carries the EPS 352 biosynthetic genes. Current methods used to estimate plasmid size are not exact. For instance, the perceived size of a plasmid may be effected by the degree of relaxation of the plasmid and the degree to which proteins may be associated with the plasmid. Thus, the EPS 352 plasmid is believed to be about 32 kb in size, and may be, for example, from 30 to 38 kb in size. Several research groups have linked EPS biosynthesis with plasmids of various sizes: 6.8 kb, 25.8 kb, 28 kb, 40.2 kb, and 45.5 kb (Vescovo et al., *Biotech. Letters II* **10**:709-712, 1989; Neve et al., *Biochimie* **70**:437-442, 1988; Vedamuthu et al., *Appl. Environ. Microbiol.* **51**:677-682, 1986; Kranenburg et al. *Mol. Microbiol.* **24**:387-397, 1997; and Von Wright et al., *Appl. Environ. Microbiol.* **53**:1385-1386, 1987).

Food means any eatable or drinkable substance consumed by humans or animals, e.g., milk, cream, dairy products, soy products, fruit juice, vegetable juices, ice cream, soups, etc.

Food Product means any food that is produced by altering its original state, e.g., milk to which has been added EPS 352.

Milk is used broadly herein to include all dairy products regardless of fat content or lactose content. The term as used herein also includes substances commonly used in place of milk, such as soy used as "soy milk". The term also includes milk products from animals other than cows, including goat milk.

Liquid as used herein includes fluids with varying degrees of fluidity including highly fluid liquids such as non-fat milk, thicker liquids such as full fat milk and cream, semi-solid substances, and gels such as yogurt and other fermented milk products. A liquid may be altered from its original state to produce an altered liquid, e.g., an adhesive solution, a paint emulsion, a lubricant, or a fruit juice to which EPS 352 has been added.

A Milk-Based liquid is any liquid wherein milk forms an appreciable percentage of the total volume of the liquid. For example, a liquid having 0.10% or more of milk solids.

A Soy-Based liquid is any liquid wherein soy forms an appreciable percentage of the total volume of the liquid. For example, a liquid having 0.10% or more of soy solids

To Thicken means to decrease fluidity and **increase viscosity.**

Thickener means any substance used to thicken, including, for instance, exopolysaccharides. A thickener may be produced by organisms cultured within a medium or may be added exogenously to a medium.

Mouth-feel is a term of art used in the food industry to describe sensory characteristics of a food. It has the same meaning as the word "texture" which has been previously defined as "the composite of the structural elements of the food and the manner in which it registers with the physiological sense" (Szczesniak, *J. Food Science* **28**:385-389, 1963), or "the composite of those properties which arise from the physical structural elements and the manner in which it registers with the physiological senses" (Sherman, *J. Food Science* **27**:381-385, 1970).

Pharmaceutical a chemical compound or composition capable of inducing a desired therapeutic or prophylactic effect when properly administered to a subject.

Beauty care product is an externally applied product that is intended to alter the appearance of the subject to which it has been applied.

Coating agent an agent applied to the exterior surface of an object. A coating agent generally forms a thin layer on the surface of the object.

Transformed refers to a cell into which a nucleic acid molecule has been introduced by molecular biology techniques. The term encompasses all techniques by which a nucleic acid molecule might be introduced into such a cell, including transformation with plasmid vectors, transfection with viral vectors, and introduction of naked DNA by electroporation, lipofection, and particle gun acceleration.

Purified does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified polysaccharide preparation is one in which the subject polysaccharide is more pure than in its natural environment within a cell or within a cell culture medium. Generally, a polysaccharide preparation is purified

such that the polysaccharide represents at least 50% of the total polysaccharide content of the preparation.

Isolated an *isolated* nucleic acid has been substantially separated or purified away from other nucleic acid sequences in the cell of the organism in which the nucleic acid naturally occurs, i.e., other chromosomal and extrachromosomal DNA and RNA. The term "isolated" thus encompasses nucleic acids purified by standard nucleic acid purification methods. The term also embraces nucleic acids prepared by recombinant expression in a host cell, as well as chemically synthesized nucleic acids.

ORF is an open reading frame. An ORF is a contiguous series of nucleotide triplets coding for amino acids. These sequences are usually translatable into a peptide.

Operably linked means a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in the same reading frame.

Probe is an isolated nucleic acid attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes.

Target Nucleic Acid is a nucleic acid that hybridizes with a probe. The conditions under which hybridization occurs may vary with the size and sequence of the probe and the target sequence.

By way of illustration, only a hybridization experiment may be performed by hybridization of a DNA probe (for example, a probe derived from the EPS 352 plasmid labeled with a chemiluminescent agent) to a target DNA molecule which has been electrophoresed in an agarose gel and transferred to a nitrocellulose membrane by Southern blotting (a technique well known in the art and described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., vols. 1-3, Cold Spring Harbor, New York, 1989).

Hybridization with a radio-labeled probe is generally carried out in a solution of high ionic strength such as 6 x SSC at a temperature that is 20°C-25°C below the melting temperature, T_m, described below. For such Southern hybridization experiments where the target DNA molecule on the Southern blot contains 10 ng of DNA or more, hybridization is typically carried out for 6-8 hours using 1-2 ng/mL radiolabeled probe. Following hybridization, the nitrocellulose filter is washed to remove background hybridization. The wash conditions should be as stringent as possible to remove background hybridization but to retain a specific hybridization signal. The term T_m represents the temperature above which, under the prevailing ionic conditions, the radiolabeled probe molecule will not hybridize to its target DNA molecule. The T_m of such a hybrid molecule may be estimated from the following equation:

$$T_m = 81.5°C - 16.6 (\log_{10} [Na^+]) + 0.41 (\%G+C) - 0.63 (\% \text{ formamide}) - (600 / l)$$

Where l = the length of the hybrid in base pairs. This equation is valid for concentrations of Na^+ in the range of 0.01M to 0.4M, and it is less accurate for calculations of T_m in solutions of higher $[Na^+]$. The equation is primarily valid for DNAs whose G+C content is in the range of 30% to 75%, and applies to hybrids greater than 100 nucleotides in length (the behavior of oligonucleotide probes is described in detail in Ch. 11 of Sambrook et al., 1989).

Generally hybridization wash conditions are classified into categories, for example very high stringency, high stringency, and low stringency. The conditions corresponding to these categories are provided below.

<u>Very High Stringency (detects sequences that share 90% sequence identity)</u>

Hybridization	in	5x	SSC	at	65°C	16 hours
Wash twice	in	2x	SSC	at	Room temp.	15 minutes each
Wash twice	in	0.2x	SSC	at	65°C	20 minutes each

High Stringency (detects sequences that share 80% sequence identity or greater)

Hybridization	in	3x	SSC	at	65°C	16 hours
Wash twice	in	2x	SSC	at	Room temp.	15 minutes each
Wash twice	in	0.5x	SSC	at	55°C	20 minutes each

Low Stringency (detects sequences that share greater than 50% sequence identity)

Hybridization	in	3x	SSC	at	65°C	16 hours
Wash twice	in	2x	SSC	at	Room temp.	20 minutes

The above example is given entirely by way of theoretical illustration. One skilled in the art will appreciate that other hybridization techniques may be utilized and that variations in experimental conditions will necessitate alternative calculations for stringency.

Conservative amino acid substitutions are those substitutions that, when made, least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids that may be substituted for an original amino acid in a protein and that are regarded as conservative substitutions.

TABLE 1

Original Residue	Conservative Substitutions
ala	ser
arg	lys
asn	gln; his
asp	glu
cys	ser
gln	asn
glu	asp
gly	pro

Original Residue	Conservative Substitutions
his	asn; gln
ile	leu; val
leu	ile; val
lys	arg; gln; glu
met	leu; ile
phe	met; leu; tyr
ser	thr
thr	ser
trp	tyr
tyr	trp; phe
val	ile; leu

Conservative substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

The substitutions which in general are expected to produce the greatest changes in protein properties will be non-conservative. For instance, changes in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histadyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

Primers are short nucleic acids, preferably DNA oligonucleotides 10 nucleotides or more in length, which are annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods known in the art.

Probes and primers as used in the present invention typically comprise at least 15 contiguous nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 30, 40, 50, 60, 70, 80, 90, 100, or 150 consecutive nucleotides of the disclosed nucleic acid sequences.

Methods for preparing and using probes and primers are described in the references, for example Sambrook et al., *Molecular Cloning: A Laboratory Manual,* 2nd ed., vol. 1-3, Cold Spring Harbor, New York, 1989; Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publ. Assoc. & Wiley-Intersciences, 1987; Innis et al., *PCR Protocols, A Guide to Methods and Applications,* 1990. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as *Primer* (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge, MA).

Recombinant nucleic acid is a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques such as those described in Sambrook et al. (1989). The term recombinant includes nucleic acids that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence. Such a recombinant nucleic acid may be part of a vector, used to transform a cell.

Sequence identity: The similarity between two nucleic acid sequences or between two amino acid sequences is expressed in terms of the level of sequence identity shared between the sequences. Sequence identity is typically expressed in terms of percentage identity; the higher the percentage, the more similar the two sequences.

Methods for aligning sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith & Waterman, *Adv. Appl. Math.* **2:**482, 1981; Needleman & Wunsch, *J. Mol. Biol.* **48:**443, 1970; Pearson & Lipman, *Proc. Natl. Acad. Sci. USA* **85:**2444, 1988; Higgins & Sharp,

Gene **73:**237-244, 1988; Higgins & Sharp, *CABIOS* **5:**151-153, 1989; Corpet et al., *Nucleic Acids Research* **16:**10881-10890, 1988; Huang, et al., *CABIOS* **8:**155-165, 1992; and Pearson et al., *Methods in Molecular Biology* **24:**307-331, 1994. Altschul et al., *J. Mol. Biol.* **215:**403-410, 1990, presents a detailed consideration of sequence alignment methods and homology calculations.

The NCBI Basic Local Alignment Search Tool (BLAST™) (Altschul et al., *J. Mol. Biol.* **215**:403-410, 1990 is available from several sources, including the National Center for Biotechnology Information (NBCI, Bethesda, MD) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. BLAST™ can be accessed on the interned at NBCI website. A description of how to determine sequence identity using this program is available at the web site. As used herein, sequence identity is commonly determined with the BLAST™ software set to default parameters. For instance, blastn (version 2.0) software may be used to determine sequence identity between two nucleic acid sequences using default parameters (expect = 10, matrix = BLOSUM62, filter = DUST (Tatusov and Lipmann, in preparation as of December 1, 1999; and Hancock and Armstrong, *Comput. Appl. Biosci.* **10**:67-70, 1994), gap existence cost = 11, per residue gap cost = 1, and lambda ratio = 0.85). For comparison of two polypeptides, blastp (version 2.0) software may be used with default parameters (expect 10, filter = SEG (Wootton and Federhen, *Computers in Chemistry* **17**:149-163, 1993), matrix = BLOSUM62, gap existence cost = 11, per residue gap cost = 1, lambda = 0.85).

For comparisons of amino acid sequences of greater than about 30 amino acids, the "Blast 2 sequences" function of the BLAST™ program is employed using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1). When aligning short peptides (fewer than around 30 amino acids), the alignment should be performed using the Blast 2 sequences function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 45%, at least 50%, at least 60%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity.

METHODS

General Methods

The present invention utilizes standard laboratory practices for the cloning, manipulation and sequencing of nucleic acids, purification and analysis of proteins and other molecular biological and biochemical techniques, unless otherwise stipulated. Such techniques are explained in detail in standard laboratory manuals such as Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor, New York, 1989; and Ausubel et al., Current Protocols in Molecular Biology, Greene Publ. Assoc. & Wiley-Intersciences, 1989. Other techniques specific to Lactococcus are discussed in the inventors' publications including: Dierksen et al., *Genetics of Streptococci, Enterococci and Lactcocci*, (Ferretti et al., eds.), 1995; Basel, *Dev. Biol. Stand* **85**:469-480, 1995; Dierksen et al., *J. Dairy Sci.*, **80**(8):1528-1536, 1997; and Knoshaug et al., *J. Dairy Sci.* **83**:633-640, 2000.

1. Growth and Characterization of the Ropy 352 organism.

The EPS 352 producing organism, *Lactococcus lactis* subspecies *cremoris* Ropy 352, was isolated, classified and deposited under the Budapest Convention as USDA-ARS-NCAUR-NRRL deposit number NRRL B-30229. Ropy 352 may be obtained on demand from the USDA-ARS-NCAUR-NRRL at Agricultural Research Service Culture Collection (NRRL), National Center for Agricultural Utilization Research (NCAUR), Agricultural Research Service (ARS), U.S. Department of Agriculture (USDA), 1815 North University Street, Peoria, IL 61604 U.S.A. Ropy 352 was streaked onto whey agar or defined lactococcal media containing glucose (DLMG) agar. Whey agar (Vedamuthu et al., *Appl. Microbiol.* **51**:677-682, 1986) made as previously described with the following modifications: yeast extract (5 g, Difco Laboratories, Detroit, MI) and sodium ß-glycerophosphate (19 g, Sigma Chemical Co., St. Louis, MO) were added to the centrifuged supernatant and the volume brought up to 600 mL. The second part of the media consisted of 15 g of agar and 3 drops of antifoam A (Sigma) in 400 mL of water. Both portions were autoclaved for 12 min, removed promptly, cooled to 50°C, mixed, and poured into sterile petri plates. DLMG agar (Molenaar et al., *J. Bacteriol.* **175**:5438-5444,

1993.) was prepared as two parts; part one consisted of the base media which was prepared in 758 mL of water, heated to dissolve the components, mixed with 10 mL of the metals, vitamins, and nucleic acid solutions and 12 mL of 20% glucose or lactose solution, filter sterilized, and heated to 55°C in a water bath. Part two consisted of 10 g of agar and 2 drops of antifoam A (Sigma) which were mixed into 200 mL of water, autoclaved, and cooled to 55°C. Part one was mixed into part two and poured into sterile petri plates. Ropy 352 was streaked onto plates and incubated at 30°C to produce macroscopic, individual, EPS 352 producing colonies of Ropy 352 (procedure described in inventors' publications listed above).

The EPS 352 may be recognized by the formation of viscous ropes greater than five mm in length originating from a whey agar or DLMG agar. Whey agar plates were incubated at 30°C for 48 h. Characteristic ropy phenotype is apparent from viscous rope greater than 5 mm formed when a colony is touched with a sterile toothpick. These ropes became visible when the colony was touched with a sterile toothpick and the toothpick was drawn away from the colony, thus, stretching the EPS 352 out. An additional way to recognize EPS 352 is by the formation of viscous ropes in liquid milk inoculated with Ropy 352 organism. Liquid milk was sterilized by steaming for 30 min and 10 mL of milk were inoculated with 0.5 mL of an overnight Ropy 352 culture. The milk was incubated for 18 hours at 30°C and visually examined for ropy EPS expression. These viscous ropes were visualized by touching the milk with a toothpick and drawing the toothpick away from the milk.

2. Purification and Characterization of EPS 352.

An individual EPS 352 producing Ropy 352 colony from a whey agar plate was picked and used to inoculate 1 L of polysaccharide production medium in a 2.8 L Fernbach flask. The medium was cultured at 30°C for 16 to 20 hours without shaking. The polysaccharide production medium consisted of 10% w/v nonfat milk in water, which was prepared by stirring 100 g dry milk powder into 1 L deionized water at room temperature for 1 hour and then sterilizing the mixture in an autoclave for 12 minutes at 120°C.

Ropy 352 culture broths were transferred to 500 mL centrifuge bottles and insoluble fractions were pelleted at 10 K *x g* for 20 minutes. Clarified supernatants

were dialyzed (6-8 kDa MWCO, Spectra/Por 1; Spectrum Laboratories, Inc., Laguna Hills, CA) against water containing 0.02% sodium azide for at least 24 hours.

An equal volume of absolute ethanol was added to the contents of the dialysis tubing and stirred in an ice bath. Ropy 352 cultures formed a precipitate of elongated ropes that were collected by centrifugation as described above. This was termed the Ropy fraction and contained EPS 352.

From 1 L of 10% nonfat milk medium, 34 mg of total polysaccharide was recovered from Ropy 352 cultures after centrifugation and dialysis. The polysaccharide responsible for the ropy characteristic (EPS 352) was purified by precipitation with 50% ethanol, followed by trichloroacetic acid (TCA) removal of residual protein. This Ropy fraction contained 10 mg of polysaccharide and was essentially protein free (<20 μg/mg in the final product). The Ropy fraction also contained 2.3 μg phosphorus/mg polysaccharide.

Compositional analysis of EPS 352 revealed a repeating structure composed of approximately 54% to 58% glucose, and 42% to 46% galactose. Compositional data suggests a novel structure for EPS 352 with glucose as the branch residue and galactose located at the end points.

The predominant sugar found in EPS 352, at 36 mol%, is (1,4)-linked glucose. The only sugar found as terminal non-reducing end groups (i.e., had a single linkage position) was galactose at 27 mol%; this quantity is indicative of a highly branched structure. A (1,4,6)-linked glucose reside was found at a concentration of 21 mol%; the three linkage sites indicate that it is a branch point in this structure. The least represented sugar was the (1,4)-linked galactose, which occurred at a concentration of 15 mol%. Results from this analysis are listed in Table 2:

Table 2
Identification of permethylated PAAN **(Peracetylated aldononitrile)** derivatives from Ropy 352 and Ropy polysaccharides

PAAN methyl sugar	Linkage site	Ropy fraction from Ropy 352 (mol%)
2,3,4,6-tetra-*O*-methyl galactose	1	27
2,3,6-tri-*O*-methyl galactose	1,4	15
2,4,6-tri-*O*-methyl galactose	1,6	n.d. (none detected)
2,3,4-tri-*O*-methyl galactose	1,6	n.d.
2,3,6-tri-*O*-methyl glucose	1,4	36
2,3,4-tri-*O*-methyl glucose	1,6	n.d.
3,4,6-tri-*O*-methyl mannose	1,2	n.d.
2,3-di-*O*-methyl glucose	1,4,6	21
3,4-di-*O*-methyl glucose	1,2,6	n.d.
2,4-di-*O*-methyl mannose	1,3,6	n.d.

The degree of phosphate protonation is shown in Figure 1. As sodium hydroxide was added to the polysaccharide solution, there was only one inflection in the titration profiles, indicating that the phosphate group in the Ropy fraction polysaccharides is in the form of a phosphodiester linkage rather than as the monoester, which would have shown 2 inflection points.

3. Viscosity of Milk Culture During 25 hour Fermentation with Ropy 352.

1 L of milk was inoculated with a single whey agar-grown colony of Ropy 352. Viscosity was measured with a Brookfield model LVTDV-I digital viscometer (Stoughton, MA) using a LV1 spindle.

The viscosity of the Ropy 352 culture reached a value of 44000 mPA-s at 24 hours, compared to an initial viscosity of 1 mPa-s (see Table 3). This data verifies the phenotypic observation that Ropy 352 culture thickens a liquid food product (milk).

Table 3
Viscosity change (in mPa-s) after 24 h.

Strain	Sample	0 h	24 h
Ropy 352	Fermented milk	1.0	44000
No cells	Milk	1.0	1.0

4. Isolation and Characterization of the Biosynthetic EPS 352 Plasmid.

The EPS 352 plasmid is a plasmid of about 32 kb in size that may be isolated from Ropy 352. A 2.2 KB fragment from the EPS 352 plasmid (Figure 2) and a 6.85 kb fragment (Figure 4) have been sequenced. These sequences encodes ORFs M and N which show homology to a class of sugar transfer enzymes (glycosyltransferases) known to be involved in EPS biosynthesis (Figure 2). Several restriction endonucleases cut this plasmid, including *EcoRI, EcoRV, HindIII, SacI, SphI, DraI, HincII, NdeI, Sau3AI,* and *SpeI.*

The EPS 352 plasmid contains all biosynthetic genes coding for the enzymes needed to make EPS 352. This was demonstrated by the following experiment. The EPS 352 plasmid, containing an erythromycin resistant encoded insertion element for selection, was isolated from a culture of Ropy 352 using DNA preparation methods as described in Knoshaug et al., *J. Dairy Science* **83**:633-640, 2000. (Ref for plasmid DNA isolation: O'Sullivan et al., *Appl Environ Microbiol.* **59**:2730-2733, 1993). This DNA was used to transform a plasmid-free nonropy lactococcal strain, MG1363 by electroporation as described (Dornan et al., *Lett. Appl. Microbiol.* **11**:62-64, 1990; Holo et al., *Appl. Environ. Microbiol.* **55**:3119-3123, 1989). Cells were grown for 24 hours in M17-glucose media supplemented with 0.3 M sucrose and 2% (MG1363) or 0.5% (Ropy352) glycine. Cells were pelleted, washed in cold 0.3 M sucrose three times, and resuspended in 200 μl of 0.3 cold M sucrose. DNA was added to the cells and the mixture was transferred to a chilled electroporation cuvette (0.2 cm gap). The cells were shocked (2.5 kV, 200 ohms, 25 μF) and resuspended in 8 mL of growth media supplemented with 0.3 M sucrose and 50 ng/mL em. Cells were allowed to recover for 1.5 hours before plating on whey agar containing 2 μg/mL em. Erythromycin resistant transformants were selected, and then screened for the ropy EPS 352 phenotype. MG1363 containing the EPS 352 plasmid was analyzed by Southern blot to verify the presence of the plasmid. The probe used was 1.6 kb long and specific to the Ropy 352 EPS ORF M and ORF N genes. Results demonstrated that the probe reacted with a 32 kb plasmid in Ropy352 (un-nicked and nicked forms) and with a 37 kb plasmid in EK356 (EPS

352 plasmid containing a 5.4 kb erythromycin resistant encoded insertion element for selection; un-nicked and nicked forms).

The southern blot analysis was additionally confirmed by testing the transformed bacteria for the Ropy phenotype. Results showed that the phenotypic carried over to the MG1363 strain.

5. Production of Food Products by Adding EPS 352 to a Food Substrate.

EPS 352 can be added to a liquid food substrate to increase viscosity and thickness of the liquid and to enhance texture and mouth-feel. Liquid food substrates may include, but are not limited to: milk (including low-fat and non-fat milk), milk-based liquids, whey-based liquids, soy-based liquids, fruit-juices, and oil-based liquids and emulsions. EPS 352 can be used to enhance the thickness and texture of, for example, yogurt, milk-shakes, fruit-juices, soy drinks, Scandinavian fermented milk products (e.g., "villi, "langfil," and "filmjolk,"), bakery fillings, dressings, sauces and gravies. EPS 352 can also be added to solid or semi-solid food substrates to enhance the texture of, for instance, frozen foods, canned foods and cheeses. Thickness of the liquid food substrate will increase in proportion to the amount of EPS 352 added. EPS 352 may be added to any liquid food substrate in an amount necessary to produce the desired consistency. Determining an amount necessary to produce a desired consistency is a simple matter of empirical experimentation.

A specific example of a food product made using EPS 352 is a thickened, non-fermented food product that has the qualities of yogurt, but without the need for fermentation. Milk (e.g., non-fat milk) can be used as a liquid food substrate to which an amount of EPS 352 can be added, sufficient to cause thickening to a desired consistency. EPS 352 may be supplied in the form of an essentially pure powder and added directly to the milk. The powder may be mixed into the milk at room temperature using conventional methods and the mixture may then be aliquoted into sealed containers and pasteurized. Such a product would be low in fat, have a yogurt-like consistency, and would not require fermentation, a step which is time-consuming, expensive and prone to microbial contamination.

6. Production of Milk-Derived Fermented Food Products by Adding a Pure Culture of the Ropy 352 Organism to a Food Substrate and Fermenting the Mixture.

Ropy 352 can be used to produce fermented food products such as yogurt (and other products as listed above). Such products are described as probiotic (this refers to organisms who are ingested, such as the LAB, which contribute to the health and balance of the human's intestinal tract thus possibly protecting against disease and improving nutrition). During fermentation, Ropy 352 produces the EPS 352 exopolysaccharide which imparts desirable qualities to certain foods. In particular, EPS 352 gives fermented milk products a very smooth, rich mouth-feel with a slightly sweet flavor.

A specific example of a fermented food product made using Ropy 352 is yogurt. Milk (e.g., either whole, 2% or non-fat milk) can be used as a liquid food substrate to which a pure culture of Ropy 352 can be added. The culture may be fermented, for instance at 30°C without shaking for 16 to 20 hours. The EPS 352 culture may be supplied in the form as an aliquot of liquid culture or an inoculum from an agar plate (such as milk or whey agar plate). Following fermentation, the fermented product may be aliquoted into sealed containers and pasteurized. A second specific example of a fermented food product made using Ropy 352 is a power shake for the elderly and diet shakes for the obese. Trade names such as Slimfast™ or Ensure™ can be used as a liquid food substrate to which a pure culture of Ropy 352 can be added. Both Slimfast™ and Ensure™ were inoculated with a culture of Ropy352 and incubated at 30°C for 24 hours, respectively. The results showed that not only did Ropy 352 thicken these products, but it also added active culture (probiotic) status.

The duration and temperature of fermentation may vary. Representative temperatures may range from about 17°C to 30°C and duration of fermentation of a batch culture may be from about 10 to 36 hours. Alternatively, fermentation may be done as a continuous culture with portions of the fermented product being periodically removed.

7. The Use of Enzymes Derived from the EPS 352 Plasmid

Enzymes derived from the EPS 352 plasmid can be used either *in vitro* or *in vivo* to produce and or modify EPS structure. Furthermore, these enzymes can be modified through the inclusion of one or more conservative amino acid substitutions, however, such conservative amino acid substituted variants will continue to maintain the same activity of the enzyme from which they are derived.

a. *in vitro*

Enzymes from the EPS 352 plasmid can be combined with other enzymes and substrates in vivo, such that an EPS is produced with the desired characteristics. *In vitro* production of an EPS involves provide the isolated enzymes that are to be used in the synthesis as well as the various substrates necessary for the production of the EPS. Detailed examples of EPS production *in vitro* are well known in the art and can be found for example in Bossia et al., *Cell Mol Biol (Noisy-le-grand)* **42**(5):737-58, 1996 and

Semino et al., *J Gen Microbiol* **139** (Pt 11):2745-56, 1993.

b. *in vivo*

The enzymes produced from the expression of ORFs, such as ORF M (SEQ ID NO: 14), ORF N (SEQ ID NO: 13), ORF O (SEQ ID NO: 9), and ORF P (SEQ ID NO: 10) that are derived from the EPS 352 plasmid can be placed under the control of heterologous control sequences. Such control sequences can be selected from constituative promoters, inducible promoters, enhancers, and various terminators. Together the control sequence(s) operably linked to the ORF is termed the "transgene". The transgene can then be transformed into a host organism that supports the production of an EPS. Upon expression of the protein from the transgene at least a portion of the EPS generated from the transformed host organism will be distinct from the non-transformed host organism.

It is also possible that the control sequences found in the EPS 352 plasmid can be used to express one of more of the ORF from the EPS 352 plasmid. Used in this way the "transgene" generated will be the result of using recombinant DNA technology to manipulate the endogenous EPS 352 plasmid such that the naturally occurring EPS 352 plasmid is not intact. Such transgenes result from the introduction of additional copies of one or more of the ORFs that are in the naturally

occurring EPS 352 plasmid. It is also possible that enzymes from other EPS producing organisms will be introduced into the EPS 352 operon such that the host cell expresses an EPS that is distinct from the Ropy 352 disclosed herein.

EXAMPLES

1. Production of a Thickened Milk Product by Adding a Pure Culture of the Ropy 352 Organism to Milk and Fermenting the Mixture.

Ropy EPS 352 was expressed on plates containing whey agar and in liquid milk. The whey agar plates were incubated at 30°C for 48 hours. Colonies were then touched with a sterile toothpick to test for Ropy EPS 352 expression. Liquid milk was sterilized by steaming for 30 minutes. 10 mL of the sterilized milk were then inoculated with 0.5 mL of an overnight pure culture of the Ropy 352 organism. The milk was incubated for 18 hours at 30°C and visually examined for coagulation and ropy EPS 352 expression. Ropiness was indicated using a sterile glass rod to pull ropes from the milk.

2. Production of a Thickened Liquid Product by Adding a Pure Culture of the Ropy 352 Organism to Power Drinks Designed for the Elderly and Diet Drinks Designed for the Obese.

Ropy 352 was grown and EPS 352 was expressed in Slim Fast™ (Slim-Fast Foods Co., West Palm Beach, Florida) chocolate diet drink and Ensure™ (Abbott Laboratories, Abbott Park, Illinois) chocolate fortified drink. Slim Fast™ and Ensure™ drinks were inoculated with Ropy 352 and incubated for 18 hours at 30°C and visually examined for coagulation and ropy EPS 352 expression. Ropiness was determined using a sterile glass rod to pull ropes from the milk, and by visually examining how the fermented liquid poured from a flask.

3. Use of the EPS 352 Plasmid to Transform Cells and to Produce EPS 352.

The EPS 352 plasmid, containing an erythromycin resistant encoded insertion element for detection, was isolated from a culture of Ropy 352 using DNA preparation methods as described in Knoshaug et al., *J. Dairy Sci.* **83**:633-640, 2000 (and as referred to in the methods section of this document). This DNA was used to

transform a plasmid-free nonropy lactococcal strain, MG1363. Erythromycin resistant transformants were selected, and then screened for the ropy EPS 352 phenotype. Those displaying the ropy EPS 352 phenotype were Gram stained to verify that Gram positive cocci were present. MG1363 containing the EPS 352 plasmid was analyzed by Southern blot to verify the presence of EPS 352 plasmid. Presence of the EPS 352 plasmid in MG1363 correlated to the acquisition of the ropy EPS 352 phenotype.

4. Use of EPS 352 as a Substitute for Xanthan Gum

Xanthan gum is a high molecular weight polysaccharide derived from *Xanthomonas Campestris*. It contains D-glucose, D-mannose, and D-glucuronic acid as the dominant hexose units. For a more detailed discussion of the composition, physical and chemical properties, preparation, etc. of xanthan gum, see the following publications: Federal Register, Vol. 34, No. 53, Mar. 19, 1969, Subchapter B, Part 121, Subpart D; Keltrol, Technical Bulletin DB No. 18, Kelco Company, Clark, New Jersey.

Xanthan gum is currently used in a variety of compounds, as is evidenced by the fact that a search of the United States Patent and Trademark Office website on the Internet for "xanthan gum" in the claims of U.S. patents that have issued since 1976 identified 1,276 patents. These patents show xanthan gum being used in sprayable cleaning compositions (U.S. patent No. 5,948,743), hair conditioning shampoo (U.S. patent No. 5948,739), ballpoint pen ink (U.S. patent No. 5,925,175), time-specific controlled release dosage formulations (U.S. patent No. 5,891,474), to improve gloss retention of surfactants (U.S. patent No. 5,877,142), as wells as for many other purposes.

5. Enzymatic Activity of the Enzymes Produced By the EPS 352 Plasmid

The EPS plasmid contains at least 5 previously unidentified open reading frames encoding 5 previously unidentified enzymes (O, P, N, M, and U, which are provided in SEQ ID NOS: 9, 10, 12, 13, and 14, respectively). Sequence analysis using Blast™ searching indicates that the "M" enzyme (SEQ ID NO: 13) is a glycosyltransferase enzyme. Methods of testing glycosyltransferase activity are

well known in the art and described in: van Kranenburg et al., *J. Bacteriol.* **181**(1):338-340, 1999; Kranenburg et al., *J. Bacteriol.* **181**(11):6347-6353, 1999; Stingele et al., *J. Bacteriol.* **181**(20):6354-6360, 1999; Kolkman et al., *J. Bacteriol.* **178**(13):3736-3741 1996; Kolkman et al., *J. Biol. Chem.* **272**(31):19502-19508; Breton, et al., *Curr. Opin. Struct. Biol.* **9**:563-571, 1999; and Griffiths et al., *J. Biol. Chem.* **273**(19):11752-11757, 1998, which are herein incorporated by reference.

Similarly, sequence analysis using Blast™ searching indicates that the "P" enzyme (SEQ ID NO: 10) is a polysaccharide polymerase. Methods of testing polysaccharide polymerase activity are well known in the art and described in: Gonzalez et al., *Proc.Natl. Acad. Sci.* **95**:13477-13482, 1998; Stevenson et al., *J. Bacteriol.* **178**(16):4885-4893, 1996; and Glucksmann et al., *J. Bacteriol.* **175**(21):7045-7055, 1993, which are herein incorporated by reference.

Sequence analysis using Blast™ searching indicates that the "N" enzyme (SEQ ID NO: 12) is a galactosyltransferase enzyme. Methods of testing galactosyltransferase activity are well known in the art and described in: van Kranenburg et al., *J. Bacteriol.* **181**(1):338-340, 1999; Kranenburg et al., *J. Bacteriol.* **181**(11):6347-6353, 1999; Stingele et al., *J. Bacteriol.* **181**(20):6354-6360, 1999; Kolkman et al., *J. Bacteriol.* **178**(13):3736-3741, 1996; Kolkman, et al., *J. Biol. Chem.* **272**(31):19502-19508, 1997; Breton et al., *Curr. Opin. Struct. Biol.* **9**:563-571, 1999; and Griffiths et al., *J. Biol. Chem.* **273**(19):11752-11757, 1998, which are herein incorporated by reference.

Sequence analysis using Blast™ searching indicates that the "O" enzyme (SEQ ID NO: 9) is a multi-unit transporting or exporter enzyme. Methods of testing activity are well known in the art and described in: Stevenson et al., *J. Bacteriol.* **178**(16):4885-4893, 1996; Glucksmann et al., *J. Bacteriol.* **175**(21):7045-7055, 1993; and Smith et al., *Mol. Microbiol.* **4**(11):1863-1869, 1990, which are herein incorporated by reference.

Finally, sequence analysis using Blast™ searching indicates that the "U" enzyme (SEQ ID NO: 15) is a glycosyltransferase/exporter enzyme. Methods of

testing glycosyltransferase/exporter activity are well known in the art and described in: Stevenson et al., *J. Bacteriol.* **178**(16):4885-4893, 1996; Glucksmann et al., *J. Bacteriol.* **175**(21):7045-7055, 1993; Smith et al., *Mol. Microbiol.* **4**(11):1863-1869, 190; van Kranenburg et al., *J. Bacteriol.* **181**(1):338-340, 1999; Kranenburg et al., *J. Bacteriol.* **181**(11):6347-6353, 1999; Stingele et al., *J. Bacteriol.* **181**(20):6354-6360, 1999.; Kolkman et al., *J. Bacteriol.* **178**(13):3736-3741, 1996; Kolkman et al., *J. Biol. Chem.* **272**(31):19502-19508, 1997; Breton et al., *Struct. Biol.* **9**:563-571, 1999; and Griffiths et al., *J. Biol. Chem.* **273**(19):11752-11757, 1998, which are herein incorporated by reference.

Having illustrated and described the principles of the invention in multiple embodiments and examples, it should be apparent to those skilled in the art that the invention can be modified in arrangement and detail without departing from such principles. The invention encompasses all modifications coming within the spirit and scope of the following claims.

CLAIMS

What is claimed is:

1. An isolated bacterium having the characteristics of *Lactococcus lactis* subspecies *cremoris* Ropy 352, as deposited with the USDA-ARS-NCAUR-NRRL as deposit accession number NRRL B-30229.

2. A purified ropy polysaccharide wherein the polysaccharide has characteristics comprising:

Composition:	Glucose: range of 54% to 58%	
	Galactose: range of 42% to 46%	
Charged:	Yes	
Molecular weight:	range of 800,000 to 8,000,000	
Phosphorous:	Present in backbone or sidechain	
Structure:	endpoints: galactose;	
	branchpoints: glucose	

3. A purified ropy polysaccharide, isolated from *Lactococcus lactis* subspecies *cremoris* Ropy 352.

4. The purified polysaccharide of claim 3 wherein the polysaccharide has the characteristics of:

Composition:	Glucose: range of 54% to 58%	
	Galactose: range of 42% to 46%	
Charged:	Yes	
Molecular weight:	range of 800,000 to 8,000,000	
Phosphorous:	Present in backbone or sidechain	
Structure:	endpoints: galactose;	
	branchpoints: glucose	

5. A method of thickening a liquid comprising adding to a liquid the purified polysaccharide of claim 2.

6. The method of claim 5 wherein the liquid is a food.

7. The method of claim 6 wherein the food is selected from the group consisting of milk, a milk-based liquid, a whey-based liquid, a soy-based liquid, and a fruit-juice.

8. A food product made by the method of claim 6.

9. A method of thickening a liquid comprising adding to a liquid the purified polysaccharide of claim 3.

10. The method of claim 9 wherein the liquid is a food.

11. The method of claim 10 wherein the food is selected from the group consisting of milk, a milk-based liquid, a whey-based liquid, a soy-based liquid, and a fruit-juice.

12. A food product made by the method of claim 10.

13. A method of making a food product comprising addition of a culture of Ropy 352 to a food that is devoid of Ropy 352.

14. The method of claim 10 wherein the food is selected from the group consisting of milk, a milk-based liquid, a whey-based liquid, a soy-based liquid, and a fruit-juice.

15. A food product made by the method of claim 13.

16. An isolated plasmid of approximately 20 kb derived from *Lactococcus lactis* subspecies *cremoris* Ropy 352, wherein the plasmid, when expressed in the transformed lab strain of *Lactococcus* MG1363, expresses a ropy polysaccharide, wherein the polysaccharide has characteristics comprising:

Composition:	Glucose: range of 54% to 58%
	Galactose: range of 42% to 46%
Charged:	Yes
Molecular weight:	range of 800,000 to 8,000,000
Phosphorous:	Present in backbone or sidechain
Structure:	endpoints: galactose;
	branchpoints: glucose

17. A probe comprising a detectable label attached to a nucleic acid selected from the group consisting of:

a portion of the plasmid of claim 16, and

the plasmid of claim 16.

18. A method of detecting a target nucleic acid comprising the steps of:

contacting the target nucleic acid with the probe of claim 17 under conditions wherein the probe hybridizes with the target nucleic acid, and

detecting the detectable label.

19. A cell transformed with the plasmid of claim 16.

20. The cell of claim 19, wherein the cell is selected from the group consisting of: a bacterial cell, a yeast cell, a fungal cell, an animal cell and a plant cell.

21. A method of making a food product comprising addition of the cell of claim 16 to a food that is devoid of the plasmid of claim 16.

22. A method for making a pharmaceutical product comprising:

combining an active ingredient and the purified ropy polysaccharide of claim 2.

23. A pharmaceutical product made by the method of claim 22.

24. A method of making a beauty care product, comprising adding the purified ropy polysaccharide of claim 2.

25. A beauty care product made by the method of claim 24.

26. A method of making a coating agent, comprising adding the purified ropy polysaccharide of claim 2.

27. A coating agent made by the method of claim 26.

28. A purified protein, comprising an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence selected from the group consisting of SEQ ID NOS: 9, 10, 13, 14, and 16;

(b) an amino acid sequence that differs from those specified in (a) by one or more conservative amino acid substitutions; and

(c) an amino acid sequence having at least 60% sequence identity to the sequences specified in (a).

29. An isolated nucleic acid molecule encoding a protein according to claim 28.

30. An isolated nucleic acid molecule, comprising a nucleic acid sequence selected from the group consisting of:

(a) a nucleic acid sequence selected encoding an amino acid sequence selected from the group consisting of: SEQ ID NOS: 9, 10, 13, 14, and 15;

(b) a nucleic acid sequence that shares at least 60% sequence identity with the nucleic acid sequences described in (a);

(b) an nucleic acid sequence that comprises at least 15 consecutive nucleotides of the sequences shown in (b).

31. A recombinant nucleic acid molecule comprising a promoter sequence operably linked to a nucleic acid sequence according to claim 30.

32. A cell transformed with a recombinant nucleic acid molecule according to claim 31.

33. A transgenic bacteria comprising a recombinant nucleic acid according to claim 31.

34. A method of producing a protein, comprising:

culturing a cell according to claim 32, wherein the cell expresses at least one protein from the recombinant nucleic acid; and

isolating the protein.

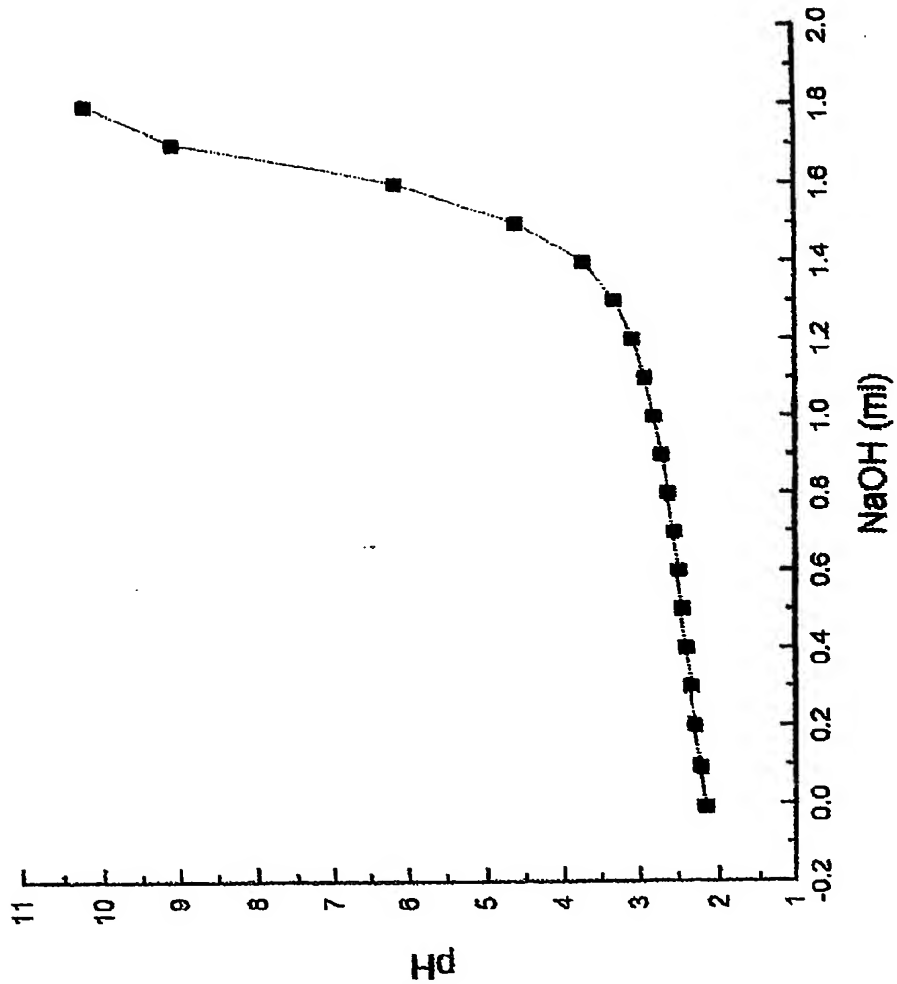

Figure 1

Sequence of Two Genes (EpsM and EpsN) Necessary for EPS352 Expression
(2265 base pairs from the start of EpsM to the end of EpsN)

```
gtctctcttttaataaatttttcccttgaattaattcgaattttaacccctcatattttaactcgcttttaaattagtcataatatcatggtcaaatattaagtctt
cagagagaaaattatttaaaaagggaacttaattaagcttaaaattggggagtataaaattgagcgaaaatttaatcagtattatagtaccagtttataattcagaa
 Q  R  E  N  Y  L  K  R  E  L  N  -  A  -  N  W  G  S  I  K  L  S  E  N  L  I  S  I  I  V  P  V  Y  N  S  E

ttcataaattctcgccgataagtatcagataatttagtttgaatagttttataacttcaataaaactaattactacccaggtgactaccgagtgttctcgattaatcg
aagtatttaagagcggctattcatagtctattaaatcaaacttatcaaaatattgaagttattttgattaatgatgggtccactgatggctcacaagagctaattagc
 K  Y  L  R  A  A  I  H  S  L  L  N  Q  T  Y  Q  N  I  E  V  I  L  I  N  D  G  S  T  D  G  S  Q  E  L  I  S

agtaaagtttttttcctatttcttaatttaatatattatgattttagacccccatagcgtacgctctttaataccataactatctcgatcaccaagcatataatac
tcatttcaaaaaaaggataaaagaattaaattatataatactaaaaatctgggggtatcgcatgcgagaaattatggtattgatagagctagtggttcgtatattatg
 S  F  Q  K  K  D  K  R  I  K  L  Y  N  T  K  N  L  G  V  S  H  A  R  N  Y  G  I  D  R  A  S  G  S  Y  I  M

aaaaatctgggtctgctgtgaatactattttcaatgacaaatctttactaacccaactaattattcaaattacgactacaacaatactcattaatgatatatacgtttt
ttttagacccagacgacacttatgataaaagttactgtttagaaatgattgggttgattaataagtttaatgctgatgttgttatgagtaattactatatatgcaaa
 F  L  D  P  D  D  T  Y  D  K  S  Y  C  L  E  M  I  G  L  I  N  K  F  N  A  D  V  V  M  S  N  Y  Y  I  C  K

ccgtttttatatataggattacaattattactagaagaacttacacttccggaggatagttccctattttgttacgcaagttatgatagactatgtccaaaatttccc
ggcaaaaatatatatcctaatgttaataatgatcttcttgaatgtgaaggcctcctatcaagggataaaacaatgcgttcaatactatctgatacaggttttaaaggg
 G  K  N  I  Y  P  N  V  N  N  D  L  L  E  C  E  G  L  L  S  R  D  K  T  M  R  S  I  L  S  D  T  G  F  K  G

aaacatacctgttcttaaaaatctttttacattaattattacaatttaagttactctcgtatttaatgaatcttctgtacaataaattataatcataacatgtattat
ttgtatggacaagaatttttagaaaaatgtaattaataatgttaaattcaatgagagcataaattacttagaagacatgttatttaatattagtattgtacataat
 F  V  W  T  R  I  F  R  K  N  V  I  N  N  V  K  F  N  E  S  I  N  Y  L  E  D  M  L  F  N  I  S  I  V  H  N

cgttcttaatatcggatatgtttattttctgtaataaaaataaatgtttctcttctaagacgtagtttttttaaatcgtttagaaaaaaatttagggaattagaataa
gcaagaattatagcctatacaaataaaagacattatttttatttacaaagagaagattctgcatcaaaaaaatttagcaaatctttttttaaatcccttaatcttatt
 A  R  I  I  A  Y  T  N  K  R  H  Y  F  Y  L  Q  R  E  D  S  A  S  K  K  F  S  K  S  F  F  K  S  L  N  L  I

tctccctttcaactaggacttaaaataagcgtttaactaagacaataaaaaatattaaatcaacctaccaattattgactctctttctcatccttttatcagttaaa
agagggaaagttgatcctgaattttattcgcaaattgattctgttatttttataatttagttggatggttaataactgagagaaagagtagggaaaatagtcaattt
 R  G  K  V  D  P  E  F  Y  S  Q  I  D  S  V  I  F  Y  N  L  V  G  W  L  I  T  E  R  K  S  R  E  N  S  Q  F

tattcctctttataatttttatactttagggttcaattcaaattttgcgaattttaccttttgggttatttttaaattataattttaattcgatacgaaaagggaat
ataaggagaaatattaaaaatatgaaatcccaagttaagtttaaaacgcttaaaatggaaaacccaataaaaaatttaatattaaaattaagctatgcttttccctta
 I  R  R  N  I  K  N  M  K  S  Q  V  K  F  K  T  L  K  M  E  N  P  I  K  N  L  I  L  K  L  S  Y  A  F  P  L
```

Figure 2A

```
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gtaggatcgtgtatgatacatatgttatccgtttttatgaaaaccaaactttattccaaattaatgagtatgttaaggaaagggtgaatcaaaaacaatatttaagat
 V  G  S  C  M  I  H  M  L  S  V  F  M  K  T  K  L  Y  S  K  L  M  S  M  L  R  K  G  -  I  K  N  N  I  -  D

tttaaaaccccaattttggttaagacacccaacctgtatgtaatttagatttcgtaaaaattacgctcagaactggcaccagtatcccctaaactgaagattcttaca
aaattttggggttaaaaccaattctgtgggttggacatacattaaatctaaagcatttttaatgcgagtcttgaccgtggtcataggggatttgacttctaagaatgt
 K  F  W  G  -  N  Q  F  C  G  L  D  I  H  -  I  -  S  I  F  N  A  S  L  D  R  G  H  R  G  F  D  F  -  E  C

acaattcgtaatgattgcctcaatcttaaaatctctcgcattttatagaacactattaataattgaatagttcatgtctggttttatgacctcaaattgtccttgaca
tgttaagcattactaacggagttagaattttagagagcgtaaaatatcttgtgataattattaacttatcaagtacagaccaaaatactggagtttaacaggaactgt
 C  -  A  L  L  T  E  L  E  F  -  R  A  -  N  I  L  -  -  L  L  T  Y  Q  V  Q  T  K  I  L  E  F  N  R  N  C


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tagaatataattttatataattaggagtagaataaagagatgaatccattaatatcaattattgttccaatatacaatgttgagaagtatattggtagtttagtaaat
 -  N  I  I  L  Y  N  -  E  -  N  K  E  M  N  P  L  I  S  I  I  V  P  I  Y  N  V  E  K  Y  I  G  S  L  V  N

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 S  L  L  K  Q  T  N  K  N  F  E  V  I  F  I  D  D  G  S  T  D  E  S  M  Q  I  L  K  E  I  M  A  G  S  E  Q

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gaattttcgttcaagttgttgcaacaagttaatcagggtttatcttcagccaggaatatcggtatacttaatgcaactggagaatatatctttttttttggattcagat
 E  F  S  F  K  L  L  Q  Q  V  N  Q  G  L  S  S  A  R  N  I  G  I  L  N  A  T  G  E  Y  I  F  F  L  D  S  D

ctactttatctttcgttaaaacacctctgttaaaactgatcaacgatatttatgtcagttggcctatgtgaatagaaactaatatcatcgtaactacttaaaccttta
gatgaaatagaaagcaattttgtggagacaattttgactagttgctataaatacagtcaaccggatacacttatctttgattatagtagcattgatgaatttggaaat
 D  E  I  E  S  N  F  V  E  T  I  L  T  S  C  Y  K  Y  S  Q  P  D  T  L  I  F  D  Y  S  S  I  D  E  F  G  N

cgaaacctgtcattaatacccgtaccttcataaatagcagtttttctaaacacatgttcactcgtttataattgacgtaacagatttctactctatggttgttgacgt
gctttggacagtaattatgggcatggaagtatttatcgtcaaaaagatttgtgtacaagtgagcaaatattaactgcattgtctaaagatgagataccaacaactgca
 A  L  D  S  N  Y  G  H  G  S  I  Y  R  Q  K  D  L  C  T  S  E  Q  I  L  T  A  L  S  K  D  E  I  P  T  T  A

accagtaaacattgttttgcgagacactaactttttgtgctaaatgataaaagacaacctttttttaaacttctattgttaaaatgcggctttcaaaaaatgaaatca
tggtcatttgtaacaaaacgctctgtgattgaaaaacacgatttactatttctgttggaaaaaaatttgaagataacaattttacgccgaaagtttttttactttagt
 W  S  F  V  T  K  R  S  V  I  E  K  H  D  L  L  F  S  V  G  K  K  F  E  D  N  N  F  T  P  K  V  F  Y  F  S
```

Figure 2B

tttttgtaacaacaataaagggattctaacatatctatatcctttgcgagacccagataatactcattagcgggccttttttaagaaaagcctgctgcggtaaaaacat
aaaaacattgttgttatttccctaagattgtatagatataggaaacgctctgggtctattatgagtaatcgcccggaaaaattcttttcggacgacgccatttttgta
K N I V V I S L R L Y R Y R K R S G S I M S N R P E K F F S D D A I F V

tgt * atactgaataatctaaaaatactagtcatattttaagcccttaaccctcgtcatcaaccattttatcaatactgttgtaatcgaagaaaaggtctaagcttt

aca * tatgacttattagatttttatgatcagtataaaattcgggaattgggagcagtagttggtaaaatagttatgacaacattagcttcttttccagattcgaaa

T * Y D L L D F Y D Q Y K I R E L G A V V G K I V M T T L A S F P D S K

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K L Y N E L N P I R K K V F K D Y I S I E K R H T K R I K M Y V K M Y V

aaaagaagaatacaacctatatttgaaatgtctgaccattttccatttgtgaccttcacttatattaaaaattagaataaatac
ttttcttcttatgttggatataaactttacagactggtaaaaggtaaacactggaagtgaatataatttttaatcttatttatg
F S S Y V G Y K L Y R L V K G K H W K - I - F L I L F M

* *ISS1* insertion site

Figure 2C

B. Alignments

1) Alignment of EpsM to EpsN

```
EpsM  LSENLISIIVPVYNSEKYLRAAIHSLLNQTYQNIEVILINDGSTDGSQELISSFQKKDKR---IKLYNTKNLGVSHARNYGIDRA
EpsN  -MNPLISIIVPIYNVEKYIGSLVNSLLKQTNKNFEVIFIDDGSTDESMQILKEIMAGSEQEFSFKLLQQVNQGLSSARNIGILNA
        : *******:** ***: : ::***:** :*:***:*:***** * :::..:    .::    :** :  * *:* *** ** .*

EpsM  SGSYIMFLDPDDTYDKSYCLEMIGLINKFN-ADVVMSNYYICK--GKNIYPNVNNDLLECEGLLSRDKTMRSILSDTGFKGFVWT
EpsN  TGEYIFFLDSDDEIESNFVETILTSCYKYSQPDTLIFDYSSIDEFGNALDSNYGHGSIYRQKDLCTSEQILTALSKDEIPTTAWS
      :*.**:***.**  :..:   ::    *:. .*.:: :*    .  *: : .* .:. :  :  *. .: : : **.  :   .*:

EpsM  RIFRKNVINNVKFNESIN-YLEDMLFNISIVHNARIIAYTNKRHYFYLQREDSASKKFSKSFFKSLNLIRGKVDPEFYSQIDSVI
EpsN  FVTKRSVIEKHDLLFSVGKKFEDNNFTPKVFYFSKNIVVISLRLYRYRKRSGSIMSNRPEKFFSDDAIFVTYDLLDFYDQYKIRE
       : ::.**:: .:  *:.  :**  *. .:.: :: *.  . * * * :*..*  .: .:.**..  ::      :**.* .

EpsM  FYNLVG-WLITERKSRENSQFIRRNIKNMKSQVKFKTLKMENPIKNLILKLSYAFPLVGSCMIHMLSVFMKTKLYSKLMSMLRKG
EpsN  LGAVVGKIVMTTLASFPDSEKLYNELNPIRKKVFKDYISIEKRHTKR-IKMY--------VKMYVFSSYVGYKLYRLVKGKHWK-
      :  :**  ::*   *  :*: : .::: ::.:*  . :.:*:  .:  :*:             ::::* ::  ***  : .   *
```

Figure 3A

2) Alingment of EpsM to EpsG (a *Lactococcus lactis* glycosyltransferase involved in a different EPS operon)

```
EpsM  LSENLISIIVPVYNSEKYLRAAIHSLLNQTYQNIEVILINDGSTDGSQELISSFQKKD-KRIKLYNTKNLGVSHARNYGIDRASG
EpsG  --MIKLSIIIPIYNVEKYLSKCLNSILEQTYKEIEIILVNDGSTDNSKDIAVSYCERFPNVFKYFEKDNGGLSSARNFGLEKISG
         :***:*:** ****  .::*:*:***::**:**:******.*:::  *: ::  : :* ::..* *:* ***:*::: **

EpsM  SYIMFLDPDDTYDKSYCLEMIGLINKFNADVVMSNYYICKGKNIYPNVNNDLLECEGLLSRDKTMRSILSDTGFKGFVWTRIFRK
EpsG  DFVGFLDSDDYIDNDLYEIMINSL---D-----SSIKIVECDFIWEYENGKSVLDK--TSEYNSIKDLMVNG--RVVAWNKIYNV
      .:: ***.** *:.   **. :  :   *.  * : . *:  *.. : :  *. ::::.:: :  : ..*.:*:.

EpsM  NVIN--NVKFNESINYLEDMLFNISIVHNARIIAYTNKRHYFYLQREDSASKKFSKSFFKSLNLIRGKVDPEFYSQIDSV--IFY
EpsG  EWLEKINIKFKEGLLY-EDLNFFFKIVPHLTSISEVSTVKNSFVHYVQHKGTITSDNSLNILDIIKSYEDVFHYYNEKQINDLYF
      : ::  *:**:*.: * **: * :.** :  *: ... : ::: : .. *.. :: *::*:. * .* : ..: :::

EpsM  NLVGWLITERKSRENSQFIRRNIKN-MKSQVKFKTLKMENPIKNLILKLSYAFPLVGSCMIHMLSVFMKTKLYSKLMSMLRKG
EpsG  DELEYKFSRNLMG---AFLKRAIKIKDKRQRKIILDEFWNNVLSYYPNWKKNKYIKKLSKQNILLFFINKYTY-KLFYLL---
      : : : ::..   *::* **  * * *:  :: * : .  : .  :  . ::* .*::. * **: :*
```

3) Alingment of EpsN to EpsG

```
EpsN  MNPLISIIVPIYNVEKYIGSLVNSLLKQTNKNFEVIFIDDGSTDESMQILKEIMAGSEQEFSFKLLQQVNQGLSSARNIGILNAT
EpsG  -MIKLSIIIPIYNVEKYLSKCLNSILEQTYKEIEIILVNDGSTDNSKDIAVSYCERFPN--VFKYFEKDNGGLSSARNFGLEKIS
        :***:********:.. :**:*:** *::*:*:::*****:* :*  .  :  ** ::: * *******:*: : :

EpsN  GEYIFFLDSDDEIESNFVETILTSCYKYSQPDTLIFDYSSIDEFGNALDSNYGHGSIYRQKDLCTSEQILTALSKDEIPTTAWSF
EpsG  GDFVGFLDSDDYIDNDLYEIMIN----SLDSSIKIVECDFIWEYEN------GKSVLDKTSEYNSIKDLMVNG--RVV---AWNK
      *::: ****** *:.:: * ::.   :.. *.: . * *: *   *:. : : .: : ::::.   :  **.

EpsN  VTKRSVIEKHDLLFSVGKKFEDNNFTPKVFYFSKNIVVIS---LRLYRYRKRSGSIMS----NRPEKFFSDDAIFVTYD---LLD
EpsG  IYNVEWLEKINIKFKEGLLYEDLNFFFKIVPHLTSISEVSTVKNSFVHYVQHKGTITSDNSLNILDIIKSYEDVFHYYNEKQIND
      : : . :** :: *. * :** ** *:. ..* :*  : :* ::.*:* *  * : : * : :* *:  : *

EpsN  FYDQYKIRELGAVVGKIVMTTLASFPDSEKLYNELNPIRKKVFKDYISIEK-RHTKRIKMYVKMYVFSSYVGYKLYRLVKGKHWK
EpsG  LYFDELEYKFSRNLMGAFLKRAIKIKDKRQRKIILDEFWNNVLSYYPNWKKNKYIKKLSKQNILLFFINKYTYKLFYLL------
      :* :  ::. :  .:.  .: *..:  *: : ::*:. * . :* :: *::.  : .* .  ***: *:
```

Figure 3B

Organization of pEPS352

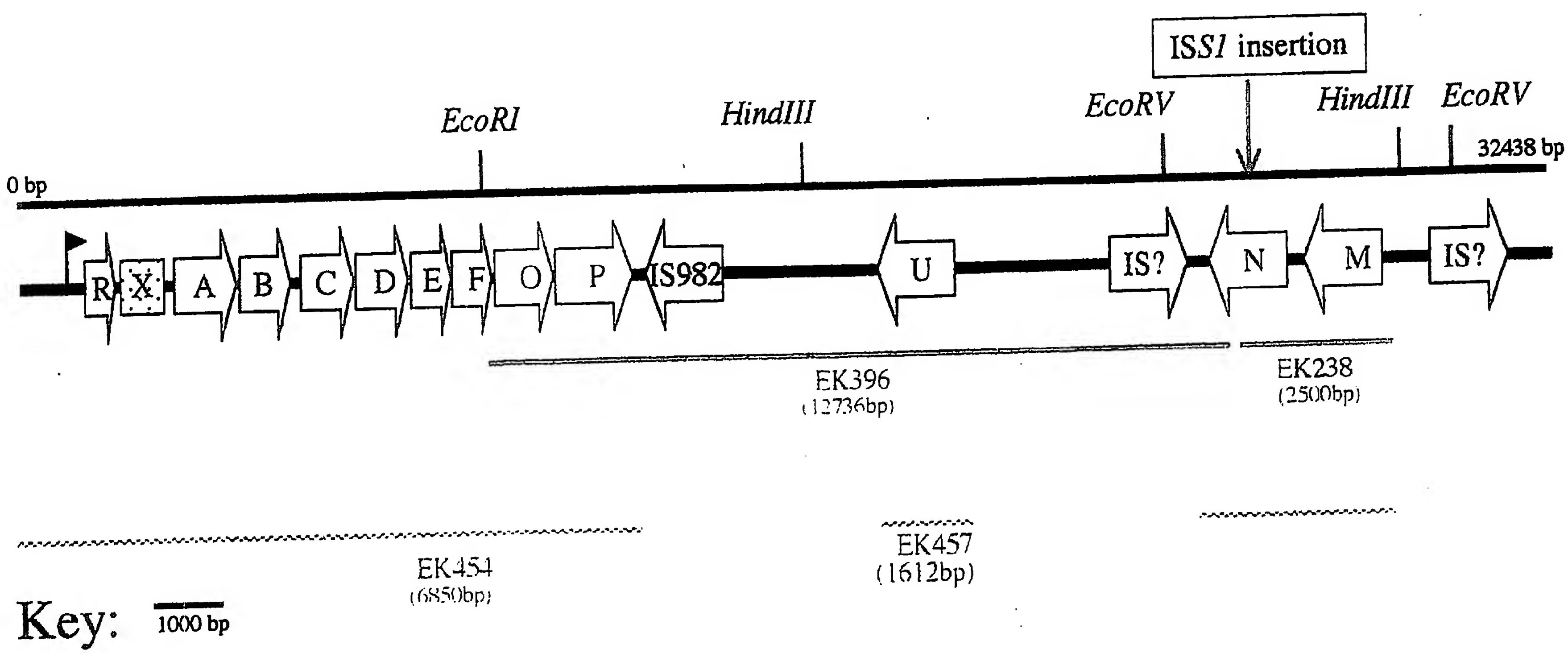

Figure 4

Eps352 Operon seqeunce EpsR-EpsK (primer EpsOPF-EpsOPR) corrected as of May8, 2000

```
GTTGAAAAACCCTACCTTTACTTGCACTAATAGGTTTTATTTTATATAATCATTGATATAATATTGAAAATTAAAAAACACCAAAATGGTTTAACTTAAG
CAACTTTTTGGGATGGAAATGAACGTGATTATCCAAAATAAAATATATTAGTAACTATATTATAACTTTTAATTTTTTGTGGTTTTACCAAATTGAATTC

CAAGTTTTGATTTAATTTTTCAGAAAAATTAAGGTTTTTCTTACAGAAGTTAATAAAAAAAGGGATTATATTTATGAATAATTTATTTTACCATCGTCTA
GTTCAAAACTAAATTAAAAAGTCTTTTTAATTCCAAAAAGAATGTCTTCAATTATTTTTTTCCCTAATATAAATACTTATTAAATAAAATGGTAGCAGAT
                                                                           M  N  N  L  F  Y  H  R  L

AAGGAACTAGTTGAATCAAGTGGTAAATCTGCAAATCAAATAGAAAGGGAATTGGGTTACCCTAGAAATTCTTTGAATAATTATAAGTTGGGAGGAGAAC
TTCCTTGATCAACTTAGTTCACCATTTAGACGTTTAGTTTATCTTTCCCTTAACCCAATGGGATCTTTAAGAAACTTATTAATATTCAACCCTCCTCTTG
 K  E  L  V  E  S  S  G  K  S  A  N  Q  I  E  R  E  L  G  Y  P  R  N  S  L  N  N  Y  K  L  G  G  E

CCTCTGGGACAAGATTAATAGGACTATCAGAGTATTTTAATGTGTCTCCAAAAATATCTGATGGGTATAATTGATGAGCCTAATGACAGTTCTGCAATTAA
GGAGACCCTGTTCTAATTATCCTGATAGTCTCATAAAATTACACAGAGGTTTTTATAGACTACCCATATTAACTACTCGGATTACTGTCAAGACGTTAATT
P  S  G  T  R  L  I  G  L  S  E  Y  F  N  V  S  P  K  Y  L  M  G  I  I  D  E  P  N  D  S  S  A  I  N

TCTTTTTAAAACTCTAACTCAAGAAGAGAAAAAAGAAATGTTTATAATTTGTCAAAAATGGCTTTTTTTAGAATATCAAATAGAGTTATAACAATAATAA
AGAAAAATTTTGAGATTGAGTTCTTCTCTTTTTTCTTTACAAATATTAAACAGTTTTTACCGAAAAAAATCTTATAGTTTATCTCAATATTGTTATTATT
 L  F  K  T  L  T  Q  E  E  K  K  E  M  F  I  I  C  Q  K  W  L  F  L  E  Y  Q  I  E  L

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TAAATCCCTCAAAAAAGCCATCACATTTTATTCAAAACCTTGTAGTTTTTATAGTGGATGTTACCGCTTTGTTCACTTGTTAATAACCGACTTTTTCAAG
                                 N  K  F  W  N  I  K  N  I  T  Y  N  G  E  T  S  E  Q  L  L  A  E  K  V

AAAATCAAGTATTGGCGACTAACCCTGATGTTGTTTTATATGAAGCTCCACTTTTTAATGATAACCAAAACATTGAAGCAACAGCCTCATGGACTAGTAA
TTTTAGTTCATAACCGCTGATTGGGACTACAACAAAATATACTTCGAGGTGAAAAATTACTATTGGTTTTGTAACTTCGTTGTCGGAGTACCTGATCATT
Q  N  Q  V  L  A  T  N  P  D  V  V  L  Y  E  A  P  L  F  N  D  N  Q  N  I  E  A  T  A  S  W  T  S  N

TGAGCAACTTATAACAAATTTGGCTAGTACAGGAGCAGAGGTGATAGTTCAACCCTCTCCACCGATTTATGGTGGTGTTGTGTACCCCGTACAAGAAGAA
ACTCGTTGAATATTGTTTAAACCGATCATGTCCTCGTCTCCACTATCAAGTTGGGAGAGGTGGCTAAATACCACCACAACACATGGGGCATGTTCTTCTT
 E  Q  L  I  T  N  L  A  S  T  G  A  E  V  I  V  Q  P  S  P  P  I  Y  G  G  V  V  Y  P  V  Q  E  E

CAGTTTAAACAATCTTTATCTACAAAGTATCCCTATATAGACTACTGGGCTAGTTACCCAGACAAAAATTCTGATGAAATGAAGGGGCTGGTTTCTGATG
GTCAAATTTGTTAGAAATAGATGTTTCATAGGGATATATCTGATGACCCGATCAATGGGTCTGTTTTTAAGACTACTTTACTTCCCCGACCAAAGACTAC
 Q  F  K  Q  S  L  S  T  K  Y  P  Y  I  D  Y  W  A  S  Y  P  D  K  N  S  D  E  M  K  G  L  V  S  D
```

Figure 5A

```
ATGGAGTATATAGAACATTAAATGCTTCGGGGAATAAGGTTTGGCTAGATTATATTACTAAATATTTTACAGCAAACTAATTAAGTTATAAATAACAATT
TACCTCATATATCTTGTAATTTACGAAGCCCCTTATTCCAAACCGATCTAATATAATGATTTATAAAATGTCGTTTGATTAATTCAATATTTATTGTTAA
D  G  V  Y  R  T  L  N  A  S  G  N  K  V  W  L  D  Y  I  T  K  Y  F  T  A  N

ATTAAATATTGGAGAAGAAATGCAGGAAACACAGGAACAGACGATTGATTTAAGAGGGATTTTTAAAATTATTCGCAAAAGGTTAGGTTTAATATTATTT
TAATTTATAACCTCTTCTTTACGTCCTTTGTGTCCTTGTCTGCTAACTAAATTCTCCCTAAAAATTTTAATAAGCGTTTTCCAATCCAAATTATAATAAA
                    M  Q  E  T  Q  E  Q  T  I  D  L  R  G  I  F  K  I  I  R  K  R  L  G  L  I  L  F

AGTGCTTTAATAGTCACAATATTAGGGAGCATCTACACATTTTTTATAGCCTCCCCAGTTTACACAGCCTCAACTCAACTTGTCGTTAAACTACCAAATT
TCACGAAATTATCAGTGTTATAATCCCTCGTAGATGTGTAAAAAATATCGGAGGGGTCAAATGTGTCGGAGTTGAGTTGAACAGCAATTTGATGGTTTAA
 S  A  L  I  V  T  I  L  G  S  I  Y  T  F  F  I  A  S  P  V  Y  T  A  S  T  Q  L  V  V  K  L  P  N

CGGAGCATTCAGCAGCCTACGCTGGAGAAGTGACCGGGAATATTCAAATGGCGAACACAATTAACCAAGTTATTGTTAGTCCAGTCATTTTAGATAAAGT
GCCTCGTAAGTCGTCGGATGCGACCTCTTCACTGGCCCTTATAAGTTTACCGCTTGTGTTAATTGGTTCAATAACAATCAGGTCAGTAAAATCTATTTCA
S  E  H  S  A  A  Y  A  G  E  V  T  G  N  I  Q  M  A  N  T  I  N  Q  V  I  V  S  P  V  I  L  D  K  V

TCAAAGTAATTTAAATCTATCTGATGGCTCTTTCCAAAAACAAGTTACAGTAGCAAATCAAACAGATTCACAAGTTATTACGCTTACTGTTAAATATTCT
AGTTTCATTAAATTTAGATAGACTACCGAGAAAGGTTTTTGTTCAATGTCATCGTTTAGTTTGTCTAAGTGTTCAATAATGCGAATGACAATTTATAAGA
  Q  S  N  L  N  L  S  D  G  S  F  Q  K  Q  V  T  V  A  N  Q  T  D  S  Q  V  I  T  L  T  V  K  Y  S

AATCCTTACATTGCACAAAAGATTGCAGACGAGACTGCTAAAATTTTTAGTTCAGATGCAGCAAAACTATTGAATGTTACTAACGTTAATATTCTATCCA
TTAGGAATGTAACGTGTTTTCTAACGTCTGCTCTGACGATTTTAAAAATCAAGTCTACGTCGTTTTGATAACTTACAATGATTGCAATTATAAGATAGGT
 N  P  Y  I  A  Q  K  I  A  D  E  T  A  K  I  F  S  S  D  A  A  K  L  L  N  V  T  N  V  N  I  L  S

AAGCAAAAGCTCAAACAACACCAATTAGTCCTAAACCTAAATTGTATTTAGCGATATCTGTTATAGCCGGACTAGTTTTAGGTTTAGCCATTGCTTTATT
TTCGTTTTCGAGTTTGTTGTGGTTAATCAGGATTTGGATTTAACATAAATCGCTATAGACAATATCGGCCTGATCAAAATCCAAATCGGTAACGAAATAA
K  A  K  A  Q  T  T  P  I  S  P  K  P  K  L  Y  L  A  I  S  V  I  A  G  L  V  L  G  L  A  I  A  L  L

GAAGGAATTATTTGATAACAAAATTAATAAAGAAGAAGATATTGAAGCTCTGGGGCTCACGGTTCTTGGTGTAACAAGCTATGCTCAAATGAGTGATTTT
CTTCCTTAATAAACTATTGTTTTAATTATTTCTTCTTCTATAACTTCGAGACCCCGAGTGCCAAGAACCACATTGTTCGATACGAGTTTACTCACTAAAA
  K  E  L  F  D  N  K  I  N  K  E  E  D  I  E  A  L  G  L  T  V  L  G  V  T  S  Y  A  Q  M  S  D  F

AATAAGAATACAAATAAAAATGGCACGCAATCGGGAACTAAGTCAAGTCCGCCTAGCGACCATGAAGTAAATAGATCATCAAAAAGGAATAAAAGATAGG
TTATTCTTATGTTTATTTTTACCGTGCGTTAGCCCTTGATTCAGTTCAGGCGGATCGCTGGTACTTCATTTATCTAGTAGTTTTTCCTTATTTTCTATCC
 N  K  N  T  N  K  N  G  T  Q  S  G  T  K  S  S  P  P  S  D  H  E  V  N  R  S  S  K  R  N  K  R

AGTTCAGGATGGCTAAAAATAAAAGAAGCATAGACAACAATCGTTATATTATTACCAGTGTCAATCCTCAATCACCTATTTCCGAACAATATCGTTCGAT
TCAAGTCCTACCGATTTTTATTTTCTTCGTATCTGTTGTTAGCAATATAATAATGGTCACAGTTAGGAGTTAGTGGATAAAGGCTTGTTATAGCAAGCTA
          M  A  K  N  K  R  S  I  D  N  N  R  Y  I  I  T  S  V  N  P  Q  S  P  I  S  E  Q  Y  R  S  I
```

Figure 5B

```
TCGTACGACCATTGATTTTAAAATGGCGGATCAAGGAATTAAAAGTTTTCTAGTAGCATCTTCAGAAGTAGCTGTAGGTAAATCAACCGTATGTGCTAAT
AGCATGCTGGTAACTAAAATTTTACCGCCTAGTTCCTTAATTTTCAAAAGATCATCGTAGAAGTCTTCATCGACATCCATTTAGTTGGCATACACGATTA
 R  T  T  I  D  F  K  M  A  D  Q  G  I  K  S  F  L  V  A  S  S  E  V  A  V  G  K  S  T  V  C  A  N

ATAGCTGTTGCTTTTGCACAACAAGGTAAAAAAGTACTTTTAATTGATGGCGATCTTCGTAAACCGACTGTTAACATTACTTTTAAAGTACAAAATAGAG
TATCGACAACGAAAACGTGTTGTTCCATTTTTTCATGAAAATTAACTACCGCTAGAAGCATTTGGCTGACAATTGTAATGAAAATTTCATGTTTTATCTC
 I  A  V  A  F  A  Q  Q  G  K  K  V  L  L  I  D  G  D  L  R  K  P  T  V  N  I  T  F  K  V  Q  N  R

TAGGATTAACCAATATTTTAATGCATCAATCTTCGATTGAAGATGCCATACAAGGGACAAGACTTTCTGAAAATCTTACAATAATTACCTCTGGTCCAAT
ATCCTAATTGGTTATAAAATTACGTAGTTAGAAGCTAACTTCTACGGTATGTTCCCTGTTCTGAAAGACTTTTAGAATGTTATTAATGGAGACCAGGTTA
V  G  L  T  N  I  L  M  H  Q  S  S  I  E  D  A  I  Q  G  T  R  L  S  E  N  L  T  I  I  T  S  G  P  I

TCCACCTAATCCATCGGAATTATTAGCATCTAGTGCAATGAAGAATTTGATTGACTCTGTGTCCGATTTATTTGATGTTGTTTTGATTGATACTCCAACT
AGGTGGATTAGGTAGCCTTAATAATCGTAGATCACGTTACTTCTTAAACTAACTGAGACACAGGCTAAATAAACTACAACAAAACTAACTATGAGGTTGA
 P  P  N  P  S  E  L  L  A  S  S  A  M  K  N  L  I  D  S  V  S  D  L  F  D  V  V  L  I  D  T  P  T

CTCTCTGCAGTTACTGATGCTCAAATTTTGAGTAGTTATGTAGGAGGAGCAGTTATTGTTGTACGTGCCTATGAAACAAAAAAAGAGAGTTTAGCAAAAA
GAGAGACGTCAATGACTACGAGTTTAAAACTCATCAATACATCCTCCTCGTCAATAACAACATGCACGGATACTTTGTTTTTTTCTCTCAAATCGTTTTT
 L  S  A  V  T  D  A  Q  I  L  S  S  Y  V  G  G  A  V  I  V  V  R  A  Y  E  T  K  K  E  S  L  A  K

CAAAAAAAATGCTTGAACAAGTTAATACAAATATTTTAGGGGTTGTTTTGCATGGGGTAAACTCTTCTGAGTCACCATCGTATTACTACCACGGAGTAGA
GTTTTTTTTACGAACTTGTTCAATTATGTTTATAAAATCCCCAACAAAACGTACCCCATTTGAGAAGACTCAGTGGTAGCATAATGATGGTGCCTCATCT
T  K  K  M  L  E  Q  V  N  T  N  I  L  G  V  V  L  H  G  V  N  S  S  E  S  P  S  Y  Y  Y  H  G  V  E

GTAATTGGAATAAACTTGAATCAAATAAAAGACAGAAATTTGTAGAAGAGGAGAGCAAATGATTGATATTCATTGCCATATTTTACTGGAGCTAAAACTT
CATTAACCTTATTTGAACTTAGTTTATTTTCTGTCTTTAAACATCTTCTCCTCTCGTTTACTAACTATAAGTAACGGTATAAAATGACCTCGATTTTGAA

CTGGAGATACTTTGACAATGCTGAAATCAGCAATTGATGAAGGGATAACAACCATCACTGCCACTCCTCATCATAATCCTCAATTTAATAATGAATCACC
GACCTCTATGAAACTGTTACGACTTTAGTCGTTAACTACTTCCCTATTGTTGGTAGTGACGGTGAGGAGTAGTATTAGGAGTTAAATTATTACTTAGTGG
                    M  L  K  S  A  I  D  E  G  I  T  T  I  T  A  T  P  H  H  N  P  Q  F  N  N  E  S  P

GCTTATTTTGAAGAAAGTTAAGGAAGTTCAAAATATCATTGACGAGCATCAATTACCAATTGAAGTTTTACCAGGACAAGAGGTGAGAATATATGGTGAT
CGAATAAAACTTCTTTCAATTCCTTCAAGTTTTATAGTAACTGCTCGTAGTTAATGGTTAACTTCAAAATGGTCCTGTTCTCCACTCTTATATACCACTA
 L  I  L  K  K  V  K  E  V  Q  N  I  I  D  E  H  Q  L  P  I  E  V  L  P  G  Q  E  V  R  I  Y  G  D

TTATTAAAAGAATTTTCTGAAGGAAAGTTACTGACAGCAGCGGGCACTTCAAGTTATATATTGATTGAATTTCCATCAAATCATGTGCCAGCTTATGCTA
AATAATTTTCTTAAAAGACTTCCTTTCAATGACTGTCGTCGCCCGTGAAGTTCAATATATAACTAACTTAAAGGTAGTTTAGTACACGGTCGAATACGAT
 L  L  K  E  F  S  E  G  K  L  L  T  A  A  G  T  S  S  Y  I  L  I  E  F  P  S  N  H  V  P  A  Y  A
```

Figure 5C

```
AAGAACTTTTTATAATATTCAATTGGAGGGACTTCAACCTATTTTGGTCCACCCTGAGCGTAATAGCGGAATCATTGAGAACCCTGATATATTATTTGA
TTCTTGAAAAAATATTATAAGTTAACCTCCCTGAAGTTGGATAAAACCAGGTGGGACTCGCATTATCGCCTTAGTAACTCTTGGGACTATATAATAAACT
K  E  L  F  Y  N  I  Q  L  E  G  L  Q  P  I  L  V  H  P  E  R  N  S  G  I  I  E  N  P  D  I  L  F  D

TTTTATTGAACAAGGAGTACTAAGTCAGATAACAGCTTCAAGTGTCACTGGTCATTTTGGTAAAAAAATACAAAAGCTGTCATTTAAAATGATAGAAAAC
AAAATAACTTGTTCCTCATGATTCAGTCTATTGTCGAAGTTCACAGTGACCAGTAAAACCATTTTTTTATGTTTTCGACAGTAAATTTTACTATCTTTTG
 F  I  E  Q  G  V  L  S  Q  I  T  A  S  S  V  T  G  H  F  G  K  K  I  Q  K  L  S  F  K  M  I  E  N

CATCTTACGCATTTTGTTGCATCAGATGCGCATAATGTGACGTCACGTGCATTTAAGATGAAGGAAGCGTTTGAAATTATTGAAGATAGTTATGGTTCTG
GTAGAATGCGTAAAACAACGTAGTCTACGCGTATTACACTGCAGTGCACGTAAATTCTACTTCCTTCGCAAACTTTAATAACTTCTATCAATACCAAGAC
 H  L  T  H  F  V  A  S  D  A  H  N  V  T  S  R  A  F  K  M  K  E  A  F  E  I  I  E  D  S  Y  G  S

ATGTATCACGAATGTTTCAAAATAATGCAGAGTCAGTGATTTTAAACGAAAGTTTTTATCAAGAAAAACCAACAAAGATCAAAACAAAGAAATTTTTAGG
TACATAGTGCTTACAAAGTTTTATTACGTCTCAGTCACTAAAATTTGCTTTCAAAAATAGTTCTTTTTGGTTGTTTCTAGTTTTGTTTCTTTAAAAATCC
D  V  S  R  M  F  Q  N  N  A  E  S  V  I  L  N  E  S  F  Y  Q  E  K  P  T  K  I  K  T  K  K  F  L  G

ATTATTTTAAAAGGATTAAAAGGAGTAAATAATGGAATTTTTTGAGGATGCCTCATCACCTGAATCGGGAGAGCCTAAGTTAGTAGAATTAAAAAATTTT
TAATAAAATTTTCCTAATTTTCCTCATTTATTACCTTAAAAAACTCCTACGGAGTAGTGGACTTAGCCCTCTCGGATTCAATCATCTTAATTTTTTAAAA
 L  F                                M  E  F  F  E  D  A  S  S  P  E  S  G  E  P  K  L  V  E  L  K  N  F

TCTTATAGAGAGCTAATTATAAAAAGAGCAATTGATATCCTAGGAGGATTAGCAGGTTCAGTTTTATTTCTTATTGCGGCTGCATTGCTTTATATCCCTT
AGAATATCTCTCGATTAATATTTTTCTCGTTAACTATAGGATCCTCCTAATCGTCCAAGTCAAAATAAAGAATAACGCCGACGTAACGAAATATAGGGAA
 S  Y  R  E  L  I  I  K  R  A  I  D  I  L  G  G  L  A  G  S  V  L  F  L  I  A  A  A  L  L  Y  I  P

ACAAAATGAGCTCAAAAAAAGATCAAGGGCCAATGTTCTATAAACAAAAACGCTATGGTAAAAATGGTAAAATTTTTTATATTTTGAAATTTAGAACAAT
TGTTTTACTCGAGTTTTTTTCTAGTTCCCGGTTACAAGATATTTGTTTTTGCGATACCATTTTTACCATTTTAAAAAATATAAAACTTTAAATCTTGTTA
Y  K  M  S  S  K  K  D  Q  G  P  M  F  Y  K  Q  K  R  Y  G  K  N  G  K  I  F  Y  I  L  K  F  R  T  M

GATTCTTAATGCCGAGCAGTATCTAGAACTTAATCCAGATGTTAAAGCTGCTTACCATGCCAACGGCAATAAGCTAGAAAACGATCCACGGGTAACGAAG
CTAAGAATTACGGCTCGTCATAGATCTTGAATTAGGTCTACAATTTCGACGAATGGTACGGTTGCCGTTATTCGATCTTTTGCTAGGTGCCCATTGCTTC
 I  L  N  A  E  Q  Y  L  E  L  N  P  D  V  K  A  A  Y  H  A  N  G  N  K  L  E  N  D  P  R  V  T  K

ATTGGCTCATTTATAAGACGACACTCAATTGATGAACTGCCACAATTTATCAATGTTCTTAAAGGGGATATGTCATTAGTTGGTCCAAGACCAATTCTGC
TAACCGAGTAAATATTCTGCTGTGAGTTAACTACTTGACGGTGTTAAATAGTTACAAGAATTTCCCCTATACAGTAATCAACCAGGTTCTGGTTAAGACG
 I  G  S  F  I  R  R  H  S  I  D  E  L  P  Q  F  I  N  V  L  K  G  D  M  S  L  V  G  P  R  P  I  L  L
TTTTTGAAGCGAAAGAATATGGGAAACGCCTCGCTTACTTACTCATGTGCAAACCAGGAATCACTGGTTATTGGACGACACATGGTCGAAGTAAAGTTCT
AAAAACTTCGCTTTCTTATACCCTTTGCGGAGCGAATGAATGAGTACACGTTTGGTCCTTAGTGACCAATAACCTGCTGTGTACCAGCTTCATTTCAAGA
 F  E  A  K  E  Y  G  K  R  L  A  Y  L  L  M  C  K  P  G  I  T  G  Y  W  T  T  H  G  R  S  K  V  L
```

Figure 5D

```
TTTTCCTCAACGAGCAGATTTAGAACTCTATTATCTCCAGTACCATAGCACCAAAAATGATATCAAGCTTCTAGTACTCACAATTGTACAAAGTATTAAC
AAAAGGAGTTGCTCGTCTAAATCTTGAGATAATAGAGGTCATGGTATCGTGGTTTTTACTATAGTTCGAAGATCATGAGTGTTAACATGTTTCATAATTG
 F  P  Q  R  A  D  L  E  L  Y  Y  L  Q  Y  H  S  T  K  N  D  I  K  L  L  V  L  T  I  V  Q  S  I  N

GGATCGGACGCATATTAAAAAATGAAAATAGCATTAGTAGGTTCCAGCGGTGGCCATTTGACACACCTGTATTTGTTAAAAAAGTTTTGGGAAAACGAAG
CCTAGCCTGCGTATAATTTTTTACTTTTATCGTAATCATCCAAGGTCGCCACCGGTAAACTGTGTGGACATAAACAATTTTTTCAAAACCCTTTTGCTTC
G  S  D  A  Y        M  K  I  A  L  V  G  S  S  G  G  H  L  T  H  L  Y  L  L  K  K  F  W  E  N  E

ATAGATTTTGGGTCACATTTGATAAAACAGATGCAAAATCTATATTGAAAGAAGAAAGATTTTATCCTTGTTATTATCCCACAAATAGAAATGTAAAAAA
TATCTAAAACCCAGTGTAAACTATTTTGTCTACGTTTTAGATATAACTTTCTTCTTTCTAAAATAGGAACAATAATAGGGTGTTTATCTTTACATTTTTT
D  R  F  W  V  T  F  D  K  T  D  A  K  S  I  L  K  E  E  R  F  Y  P  C  Y  Y  P  T  N  R  N  V  K  N

CACGATAAAAAATACCATTCTTGCATTTAAAATACTTAGAAAAGAAAAACCAGATTTGATTATTTCGAGTGGTGCTGCGGTAGCCGTTCCTTTTTTTTGG
GTGCTATTTTTTATGGTAAGAACGTAAATTTTATGAATCTTTTCTTTTTGGTCTAAACTAATAAAGCTCACCACGACGCCATCGGCAAGGAAAAAAAACC
 T  I  K  N  T  I  L  A  F  K  I  L  R  K  E  K  P  D  L  I  I  S  S  G  A  A  V  A  V  P  F  F  W

TTAGGTAAACTATTCGGTGCAAAGACAGTCTATATTGAAATATTTGACCGGATCGATAAACCAACCTTAACAGGAAAATTAGTTTATCCAGTTACTGATA
AATCCATTTGATAAGCCACGTTTCTGTCAGATATAACTTTATAAACTGGCCTAGCTATTTGGTTGGAATTGTCCTTTTAATCAAATAGGTCAATGACTAT
 L  G  K  L  F  G  A  K  T  V  Y  I  E  I  F  D  R  I  D  K  P  T  L  T  G  K  L  V  Y  P  V  T  D

AGTTTATAGTTCAATGGGAAGAGTTAAAAAAAGTTTACCCTAAAGCAATTAATTTAGGAGGAATTTTCTAATGATTTTTGTAACGGTTGGAACTCACGAA
TCAAATATCAAGTTACCCTTCTCAATTTTTTTCAAATGGGATTTCGTTAATTAAATCCTCCTTAAAAGATTACTAAAAACATTGCCAACCTTGAGTGCTT
K  F  I  V  Q  W  E  E  L  K  K  V  Y  P  K  A  I  N  L  G  G  I  F     M  I  F  V  T  V  G  T  H  E

CAACCATTTAATCGACTCATTCAAAAAATTGATGAACTTGTACGCGATGGTGAAATCGAAGACGATGTATTCATGCAAATTGGGTACTCAACTTATGAAC
GTTGGTAAATTAGCTGAGTAAGTTTTTTAACTACTTGAACATGCGCTACCACTTTAGCTTCTGCTACATAAGTACGTTTAACCCATGAGTTGAATACTTG
 Q  P  F  N  R  L  I  Q  K  I  D  E  L  V  R  D  G  E  I  E  D  D  V  F  M  Q  I  G  Y  S  T  Y  E

CTAAATATACTAAATGGGAAAAGTTTATTGGATATGAGACTATGGAAAGATGTATGAATGAAGCGAGTACGATTATTACTCATGGCGGACCATCTACCTA
GATTTATATGATTTACCCTTTTCAAATAACCTATACTCTGATACCTTTCTACATACTTACTTCGCTCATGCTAATAATGAGTACCGCCTGGTAGATGGAT
P  K  Y  T  K  W  E  K  F  I  G  Y  E  T  M  E  R  C  M  N  E  A  S  T  I  I  T  H  G  G  P  S  T  Y

TATGCAAGTATTACAACTAGGTAAAATTCCGATAGTTGTTCCACGGCAAATGAAATTTGATGAGCATATAAATGATCATCAACTTTGGGTAAGTAAACAG
ATACGTTCATAATGTTGATCCATTTTAAGGCTATCAACAAGGTGCCGTTTACTTTAAACTACTCGTATATTTACTAGTAGTTGAAACCCATTCATTTGTC
 M  Q  V  L  Q  L  G  K  I  P  I  V  V  P  R  Q  M  K  F  D  E  H  I  N  D  H  Q  L  W  V  S  K  Q

GTTGTGAAAAAGGGATACTCATTGATTTTGTGCGAAGATGTTGAAGACATTCTCGAAAATATTATTAGTTCCAAAATTTCAGATACCTTACAAAAAAATG
CAACACTTTTTCCCTATGAGTAACTAAAACACGCTTCTACAACTTCTGTAAGAGCTTTTATAATAATCAAGGTTTTAAAGTCTATGGAATGTTTTTTTAC
 V  V  K  K  G  Y  S  L  I  L  C  E  D  V  E  D  I  L  E  N  I  I  S  S  K  I  S  D  T  L  Q  K  N
```

Figure 5E

```
TAAATCACAACACTGAATTCATAAAATTATTCAGTGCTGAAATTTACCAGCTATTTATAAAAAGTGAGAAGATATGATACCAAAAGTAATACACTATTGC
ATTTAGTGTTGTGACTTAAGTATTTTAATAAGTCACGACTTTAAATGGTCGATAAATATTTTTCACTCTTCTATACTATGGTTTTCATTATGTGATAACG
V  N  H  N  T  E  F  I  K  L  F  S  A  E  I  Y  Q  L  F  I  K  S  E  K  I  M  I  P  K  V  I  H  Y  C

TGGTTCGGAGGGCAACCTTTACCAGAATCTGCGCTAAAATGTATTGAAAGTTGGAGAAGGTTTTGTCCAGATTATGAAATAAAACAATGGTCTGAGAAAA
ACCAAGCCTCCCGTTGGAAATGGTCTTAGACGCGATTTTACATAACTTTCAACCTCTTCCAAAACAGGTCTAATACTTTATTTTGTTACCAGACTCTTTT
 W  F  G  G  Q  P  L  P  E  S  A  L  K  C  I  E  S  W  R  R  F  C  P  D  Y  E  I  K  Q  W  S  E  K  N

ACTATGATGTAAATAAAATTCAATATATTAAGGAAGCATATCAAGAAAAAAAATTTGCTTTTGTCACGGATGTTGCAAGGCTCGATATAATTTGGAATGA
TGATACTACATTTATTTTAAGTTATATAATTCCTTCGTATAGTTCTTTTTTTTAAACGAAAACAGTGCCTACAACGTTCCGAGCTATATTAAACCTTACT
  Y  D  V  N  K  I  Q  Y  I  K  E  A  Y  Q  E  K  K  F  A  F  V  T  D  V  A  R  L  D  I  I  W  N  E

AGGCGGTATATATCTTGACACGGATGTAGAGCTTATAAAATCTCTTGATGAATTGCTGTATAATAGTTTATATTTAGGAATGGAAAGAGCTGGTAGAGTA
TCCGCCATATATAGAACTGTGCCTACATCTCGAATATTTTAGAGAACTACTTAACGACATATTATCAAATATAAATCCTTACCTTTCTCGACCATCTCAT
 G  G  I  Y  L  D  T  D  V  E  L  I  K  S  L  D  E  L  L  Y  N  S  L  Y  L  G  M  E  R  A  G  R  V

AATACGGGTTTAGGGTTTGGAGCTGAAGTAAATCATCCAATTGTGAGAGCTAATTTAGAATTGTATACTAATATTCCTTTTTCAGGCAATGATAATATAA
TTATGCCCAAATCCCAAACCTCGACTTCATTTAGTAGGTTAACACTCTCGATTAAATCTTAACATATGATTATAAGGAAAAAGTCCGTTACTATTATATT
N  T  G  L  G  F  G  A  E  V  N  H  P  I  V  R  A  N  L  E  L  Y  T  N  I  P  F  S  G  N  D  N  I  T

CTTGTGTGACCTATACGACGAATCTTTTGAAAAAATATGGTCTAAAAAACAACAATGAAATTCAACATATAGATAACGCAATAATTTTACCTACTGAATA
GAACACACTGGATATGCTGCTTAGAAAACTTTTTTATACCAGATTTTTTGTTGTTACTTTAAGTTGTATATCTATTGCGTTATTAAAATGGATGACTTAT
  C  V  T  Y  T  T  N  L  L  K  K  Y  G  L  K  N  N  N  E  I  Q  H  I  D  N  A  I  I  L  P  T  E  Y

TTTATGTCCTCTAAGTTTTGAAACAAATCGATTAAAAATAACGGAAAATACTTACTCCATCCATCACTATGATATGAGTTGGAAAGATAAGAGAGATAAA
AAATACAGGAGATTCAAAACTTTGTTTAGCTAATTTTTATTGCCTTTTATGAATGAGGTAGGTAGTGATACTATACTCAACCTTTCTATTCTCTCTATTT
 L  C  P  L  S  F  E  T  N  R  L  K  I  T  E  N  T  Y  S  I  H  H  Y  D  M  S  W  K  D  K  R  D  K

TTTTTAAGACTTAAAATACAACTTAGAAAATGGGTAGGTGATGATTTTTATGAAAAAGTTATTAAAAGAATTGGAAAATAATTATCATGAATAAAATAAC
AAAAATTCTGAATTTTATGTTGAATCTTTTACCCATCCACTACTAAAAATACTTTTTCAATAATTTTCTTAACCTTTTATTAATAGTACTTATTTTATTG
F  L  R  L  K  I  Q  L  R  K  W  V  G  D  D  F  Y  E  K  V  I  K  R  I  G  K           M  N  K  I  T

CATGACAAGAGAGATGAGAGTTATTGCCTTATGTGTCGTAATTTTAGAATATTTAAATAATACAGGATTAATTGCGTCTTCAGCATACTCTTTTAGCATG
GTACTGTTCTCTCTACTCTCAATAACGGAATACACAGCATTAAAATCTTATAAATTTATTATGTCCTAATTAACGCAGAAGTCGTATGAGAAAATCGTAC
  M  T  R  E  M  R  V  I  A  L  C  V  V  I  L  E  Y  L  N  N  T  G  L  I  A  S  S  A  Y  S  F  S  M

GCGAGTACAATCCTCTTATCCTATATCTTATTCTGTAAAAAAAGAAAAGGATTTTCTTTAAAGGAGATTATTGTACTACTAATTCCATTTATTTTTGTAG
CGCTCATGTTAGGAGAATAGGATATAGAATAAGACATTTTTTTCTTTTCCTAAAAGAAATTTCCTCTAATAACATGATGATTAAGGTAAATAAAAACATC
 A  S  T  I  L  L  S  Y  I  L  F  C  K  K  R  K  G  F  S  L  K  E  I  I  V  L  L  I  P  F  I  F  V
```

Figure 5F

```
TTTTAAATCGTGATCCTAGTAATTTCAGTTTAGGGTTAATGTGGATACTCTATTTTATGTTAAGTAAGTCGGAAATAGATTTAAAAAAAGTGATGAAAAC
AAAATTTAGCACTAGGATCATTAAAGTCAAATCCCAATTACACCTATGAGATAAAATACAATTCATTCAGCCTTTATCTAAATTTTTTTCACTACTTTTG
V  L  N  R  D  P  S  N  F  S  L  G  L  M  W  I  L  Y  F  M  L  S  K  S  E  I  D  L  K  K  V  M  K  T

ATTTTTTGTTACCTCTAGTGTTTGTTTTATTTTGACAATAGTACTTTATTTAATAATGTCTCTTAATAAAAGCTCTGATATGATAATGTGGCGTGGAGAT
TAAAAAACAATGGAGATCACAAACAAAATAAAACTGTTATCATGAAATAAATTATTACAGAGAATTATTTTCGAGACTATACTATTACACCGCACCTCTA
 F  F  V  T  S  S  V  C  F  I  L  T  I  V  L  Y  L  I  M  S  L  N  K  S  S  D  M  I  M  W  R  G  D

GCTTTTATAAATCGTATGAGTTTAGGATTTATCCAACCGAATTTTGCAATGATGAGCTTTTTAGGTATAGCGATAGCCTTATTATATTTGAGTACTGAAA
CGAAAATATTTAGCATACTCAAATCCTAAATAGGTTGGATTAAAACGTTACTACTCGAAAAATCCATATCGCTATCGGAATAATATAAACTCATGACTTT
 A  F  I  N  R  M  S  L  G  F  I  Q  P  N  F  A  M  M  S  F  L  G  I  A  I  A  L  L  Y  L  S  T  E

GACAAAGAATAACTATAATTTTTATTGCCATTGTAACTTTTATTATATTTTACTTTACTCAATCAAGAACTTCAGGATATATCTTATTTTTTATTTTGAG
CTGTTTCTTATTGATATTAAAAATAACGGTAACATTGAAAATAATATAAAATGAAATGAGTTAGTTCTTGAAGTCCTATATAGAATAAAAAATAAAACTC
R  Q  R  I  T  I  I  F  I  A  I  V  T  F  I  I  F  Y  F  T  Q  S  R  T  S  G  Y  I  L  F  F  I  L  S

TATTTTATTTGTTAGTAGTAAAAAAACTAAAAAGCAAGTTTCAAATTTTGAAAAAAGGAGCATTACAGTTTTACCACTACTTCTTTTAATCATCTCTTAT
ATAAAATAAACAATCATCATTTTTTTGATTTTTCGTTCAAAGTTTAAAACTTTTTTCCTCGTAATGTCAAAATGGTGATGAAGAAAATTAGTAGAGAATA
 I  L  F  V  S  S  K  K  T  K  K  Q  V  S  N  F  E  K  R  S  I  T  V  L  P  L  L  L  L  I  I  S  Y

TCGTTGTTAAAGTTACCTATTAATCAATACATCAATAGCTTGCTTTCTGGTCGTCTGGCGCTTTATCAAGAGATTTATTCTACATTTGGTATACATTTGA
AGCAACAATTTCAATGGATAATTAGTTATGTAGTTATCGAACGAAAGACCAGCAGACCGCGAAATAGTTCTCTAAATAAGATGTAAACCATATGTAAACT
 S  L  L  K  L  P  I  N  Q  Y  I  N  S  L  L  S  G  R  L  A  L  Y  Q  E  I  Y  S  T  F  G  I  H  L

TAGGGAATAATGATGTTAAAAATACAATGTTAGATACAGCATATCTTCAAAGTTTGCTAGCAAAAGGAATTTTGTTTACATTGTTTTTATTTGTAACTTT
ATCCCTTATTACTACAATTTTTATGTTACAATCTATGTCGTATAGAAGTTTCAAACGATCGTTTTCCTTAAAACAAATGTAACAAAAATAAACATTGAAA
I  G  N  N  D  V  K  N  T  M  L  D  T  A  Y  L  Q  S  L  L  A  K  G  I  L  F  T  L  F  L  F  V  T  F

CTTTTTCATATTTTTTCTTAAGAGAAAAACACAAACTAGGTTGCAAAGTTTAGTAATTATGATGTATTTTTTAATTGCATTTACAGAAACATCATTTTTT
GAAAAAGTATAAAAAAGAATTCTCTTTTTGTGTTTGATCCAACGTTTCAAATCATTAATACTACATAAAAAATTAACGTAAATGTCTTTGTAGTAAAAAA
 F  F  I  F  F  L  K  R  K  T  Q  T  R  L  Q  S  L  V  I  M  M  Y  F  L  I  A  F  T  E  T  S  F  F

AGGTTTGTAATTTTATTTCCAGTATTGATGGTAATAATGGATCAGAAAGAGGCTAATAAAGTAATAGAAAAGGTGGCATAGTGAGTATTAATAAAACAGA
TCCAAACATTAAAATAAAGGTCATAACTACCATTATTACCTAGTCTTTCTCCGATTATTTCATTATCTTTTCCACCGTATCACTCATAATTATTTTGTCT
 R  F  V  I  L  F  P  V  L  M  V  I  M  D  Q  K  E  A  N  K  V  I  E  K  V  A

GATTGAGGAATACAAAGTATCCGTTATAGTTCCTGTTTACAATGTAGAGG
CTAACTCCTTCTGTTTCATAGGCAATATCAAGGACAAATGTTACATCTCC
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Figure 5G

Seqeunce of EpsU (start and stop codons are underlined) 1612bp total here but 1412 from start codon to stop codon

GGTGGACAGGAGGACACAATTTTTAATCCTTCCTGTTATATAGTTTTTGTTTAATATTTTTCGGGAGGGTT
ATTAATGCAAATCGCAAAAAATTATCTTTATAATGCAATATATCAGGTCTTTATAATAATTGTGCCATTAC
TTACCATTCCTTATTTGTCAAGAATTTTGGGCCCTTCAGGTATTGGAATTAACTCATATACCAATTCTATT
GTTCAGTATTTTGTTTTATTTGGTAGTATAGGAGTCGGTTTGTATGGGAATCGTCAGATTGCCTTTGTTAG
GGATAATCAGGTCAAAATGTCTAAAGTCTTTTATGAAATATTTATTTTAAGACTATTTACAATATGTTTAG
CATATTTTTTGTTCGTTGCTTTTTTAATCATTAATGGTCAGTATCATGCATACTATTTGTCTCAATCCATT
GCTATAGTTGCAGCTGCATTTGATATCTCTTGGTTTTTTATGGGAATTGAAAATTTTAAAGTAACTGTATT
AAGAAATTTTATAGTTAAGTTACTTGCTCTATTCAGTATTTTCCTATTTGTCAAATCTTACAATGATTTGA
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TATCTCGTAAAGGTTAACTATCGTGAATTAAGGCCAATAAAGCATTTAAAGCAATCTTTAGTCATGTTTAT
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GCTCCGGCTTTTTTGATCAGTCTGATAAAATAGTTAAACTGGTTTTGGCTATTGCTACTGCAACAGGTACT
GTCATGTTGCCACGTGTTGCAAATGCCTTTGCACATAGAGAGTATAGTAAAATTAAGGAATACATGTACGC
AGGTTTTTCTTTTGTGTCGGCAATTTCGATTCCTATGATGTTTGGTCTGATAGCTATTACTCCTAAATTCG
TGCCACTTTTTTTTACATCTCAATTTAGTGATGTTATTCCTGTGTTAATGATCGAGTCAATCGCAATTATT
TTTATAGCTTGGAGCAACGCAATAGGTACTCAATATCTTTTACCAACTAATCAAAATAAGTCATATACAGT
GTCGGTGATCATTGGAGCGATAGTCAATTTAATGTTAAATATTCCACTGATTATATATCTAGGTACTGTTG
GTGCATCAATTGCAACTGTAATTTCTGAAATGTCTGTAACTGTGTATCAACTTTTTATAATTCATAAACAG
CTTAATTTGCATACACTGTTTGCGGATTTATCTAAGTATTTAATTGCAGGATTAGTGATGTTTCTAATTGT
CTTTAAAATTAGTTTGTTAACACCGACATCTTGGATATTCATTCTGTTGGAAATTACTGTGGGCATAATTA
TTTATGTTGTTTTATTAATATTTTTAAAGGCAGAAATAATTAATAAGCTAAAGTTTATTATGCATAAATAG
AGGTATGGATTTAGGTACCTGCCTTATTGAAAATAACGGTGAGTCAATGGTATTGGGCATATTTGACGCTC
ACCTTCAATTTGTTTTGGTCGACTTGATTGTAGCACAGGACAATATGTCT

Figure 6

SEQUENCE LISTING

<110> Trempy, Janine, et al.

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<151> 2000-10-16

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atattgaaaa ttaaaaaaca ccaaaatggt ttaacttaag caagttttga tttaattttt 120

cagaaaaatt aaggtttttc ttacagaagt taataaaaaa aggattata ttt atg    176
                                                            Met
                                                             1

aat aat tta ttt tac cat cgt cta aag gaa cta gtt gaa tca agt ggt   224
Asn Asn Leu Phe Tyr His Arg Leu Lys Glu Leu Val Glu Ser Ser Gly
            5                   10                  15

aaa tct gca aat caa ata gaa agg gaa ttg ggt tac cct aga aat tct   272
Lys Ser Ala Asn Gln Ile Glu Arg Glu Leu Gly Tyr Pro Arg Asn Ser
        20                  25                  30

ttg aat aat tat aag ttg gga gga gaa ccc tct ggg aca aga tta ata   320
Leu Asn Asn Tyr Lys Leu Gly Gly Glu Pro Ser Gly Thr Arg Leu Ile
    35                  40                  45

gga cta tca gag tat ttt aat gtg tct cca aaa tat ctg atg ggt ata   368
Gly Leu Ser Glu Tyr Phe Asn Val Ser Pro Lys Tyr Leu Met Gly Ile
 50                  55                  60                  65

att gat gag cct aat gac agt tct gca att aat ctt ttt aaa act cta   416
Ile Asp Glu Pro Asn Asp Ser Ser Ala Ile Asn Leu Phe Lys Thr Leu
                 70                  75                  80

act caa gaa gag aaa aaa gaa atg ttt ata att tgt caa aaa tgg ctt   464
Thr Gln Glu Glu Lys Lys Glu Met Phe Ile Ile Cys Gln Lys Trp Leu
            85                  90                  95

ttt tta gaa tat caa ata gag tta taa caataataaa tttagggagt         511
Phe Leu Glu Tyr Gln Ile Glu Leu
        100                 105

tttttcggta gtgtaa aat aag ttt tgg aac atc aaa aat atc acc tac aat 563
                  Asn Lys Phe Trp Asn Ile Lys Asn Ile Thr Tyr Asn
                              110                 115

ggc gaa aca agt gaa caa tta ttg gct gaa aaa gtt caa aat caa gta   611
Gly Glu Thr Ser Glu Gln Leu Leu Ala Glu Lys Val Gln Asn Gln Val
    120                 125                 130

ttg gcg act aac cct gat gtt gtt tta tat gaa gct cca ctt ttt aat   659
Leu Ala Thr Asn Pro Asp Val Val Leu Tyr Glu Ala Pro Leu Phe Asn
135                 140                 145                 150

gat aac caa aac att gaa gca aca gcc tca tgg act agt aat gag caa   707
Asp Asn Gln Asn Ile Glu Ala Thr Ala Ser Trp Thr Ser Asn Glu Gln
                155                 160                 165

ctt ata aca aat ttg gct agt aca gga gca gag gtg ata gtt caa ccc   755
Leu Ile Thr Asn Leu Ala Ser Thr Gly Ala Glu Val Ile Val Gln Pro
            170                 175                 180

tct cca ccg att tat ggt ggt gtt gtg tac ccc gta caa gaa gaa cag   803
Ser Pro Pro Ile Tyr Gly Gly Val Val Tyr Pro Val Gln Glu Glu Gln
        185                 190                 195
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ttt aaa caa tct tta tct aca aag tat ccc tat ata gac tac tgg gct     851
Phe Lys Gln Ser Leu Ser Thr Lys Tyr Pro Tyr Ile Asp Tyr Trp Ala
    200                 205                 210

agt tac cca gac aaa aat tct gat gaa atg aag ggg ctg gtt tct gat     899
Ser Tyr Pro Asp Lys Asn Ser Asp Glu Met Lys Gly Leu Val Ser Asp
215                 220                 225                 230

gat gga gta tat aga aca tta aat gct tcg ggg aat aag gtt tgg cta     947
Asp Gly Val Tyr Arg Thr Leu Asn Ala Ser Gly Asn Lys Val Trp Leu
                235                 240                 245

gat tat att act aaa tat ttt aca gca aac taattaagtt ataaataaca      997
Asp Tyr Ile Thr Lys Tyr Phe Thr Ala Asn
            250                 255

attattaaat attggagaag aa atg cag gaa aca cag gaa cag acg att gat  1049
                         Met Gln Glu Thr Gln Glu Gln Thr Ile Asp
                                     260                 265

tta aga ggg att ttt aaa att att cgc aaa agg tta ggt tta ata tta    1097
Leu Arg Gly Ile Phe Lys Ile Ile Arg Lys Arg Leu Gly Leu Ile Leu
            270                 275                 280

ttt agt gct tta ata gtc aca ata tta ggg agc atc tac aca ttt ttt    1145
Phe Ser Ala Leu Ile Val Thr Ile Leu Gly Ser Ile Tyr Thr Phe Phe
        285                 290                 295

ata gcc tcc cca gtt tac aca gcc tca act caa ctt gtc gtt aaa cta    1193
Ile Ala Ser Pro Val Tyr Thr Ala Ser Thr Gln Leu Val Val Lys Leu
    300                 305                 310

cca aat tcg gag cat tca gca gcc tac gct gga gaa gtg acc ggg aat    1241
Pro Asn Ser Glu His Ser Ala Ala Tyr Ala Gly Glu Val Thr Gly Asn
315                 320                 325                 330

att caa atg gcg aac aca att aac caa gtt att gtt agt cca gtc att    1289
Ile Gln Met Ala Asn Thr Ile Asn Gln Val Ile Val Ser Pro Val Ile
                335                 340                 345

tta gat aaa gtt caa agt aat tta aat cta tct gat ggc tct ttc caa    1337
Leu Asp Lys Val Gln Ser Asn Leu Asn Leu Ser Asp Gly Ser Phe Gln
            350                 355                 360

aaa caa gtt aca gta gca aat caa aca gat tca caa gtt att acg ctt    1385
Lys Gln Val Thr Val Ala Asn Gln Thr Asp Ser Gln Val Ile Thr Leu
        365                 370                 375

act gtt aaa tat tct aat cct tac att gca caa aag att gca gac gag    1433
Thr Val Lys Tyr Ser Asn Pro Tyr Ile Ala Gln Lys Ile Ala Asp Glu
    380                 385                 390

act gct aaa att ttt agt tca gat gca gca aaa cta ttg aat gtt act    1481
Thr Ala Lys Ile Phe Ser Ser Asp Ala Ala Lys Leu Leu Asn Val Thr
395                 400                 405                 410

aac gtt aat att cta tcc aaa gca aaa gct caa aca aca cca att agt    1529
Asn Val Asn Ile Leu Ser Lys Ala Lys Ala Gln Thr Thr Pro Ile Ser
                415                 420                 425
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cct aaa cct aaa ttg tat tta gcg ata tct gtt ata gcc gga cta gtt    1577
Pro Lys Pro Lys Leu Tyr Leu Ala Ile Ser Val Ile Ala Gly Leu Val
            430                 435                 440

tta ggt tta gcc att gct tta ttg aag gaa tta ttt gat aac aaa att    1625
Leu Gly Leu Ala Ile Ala Leu Leu Lys Glu Leu Phe Asp Asn Lys Ile
        445                 450                 455

aat aaa gaa gaa gat att gaa gct ctg ggg ctc acg gtt ctt ggt gta    1673
Asn Lys Glu Glu Asp Ile Glu Ala Leu Gly Leu Thr Val Leu Gly Val
    460                 465                 470

aca agc tat gct caa atg agt gat ttt aat aag aat aca aat aaa aat    1721
Thr Ser Tyr Ala Gln Met Ser Asp Phe Asn Lys Asn Thr Asn Lys Asn
475                 480                 485                 490

ggc acg caa tcg gga act aag tca agt ccg cct agc gac cat gaa gta    1769
Gly Thr Gln Ser Gly Thr Lys Ser Ser Pro Pro Ser Asp His Glu Val
                495                 500                 505

aat aga tca tca aaa agg aat aaa aga tag gagttcagg atg gct aaa aat 1820
Asn Arg Ser Ser Lys Arg Asn Lys Arg              Met Ala Lys Asn
            510                 515                              520

aaa aga agc ata gac aac aat cgt tat att att acc agt gtc aat cct    1868
Lys Arg Ser Ile Asp Asn Asn Arg Tyr Ile Ile Thr Ser Val Asn Pro
                525                 530                 535

caa tca cct att tcc gaa caa tat cgt tcg att cgt acg acc att gat    1916
Gln Ser Pro Ile Ser Glu Gln Tyr Arg Ser Ile Arg Thr Thr Ile Asp
            540                 545                 550

ttt aaa atg gcg gat caa gga att aaa agt ttt cta gta gca tct tca    1964
Phe Lys Met Ala Asp Gln Gly Ile Lys Ser Phe Leu Val Ala Ser Ser
        555                 560                 565

gaa gta gct gta ggt aaa tca acc gta tgt gct aat ata gct gtt gct    2012
Glu Val Ala Val Gly Lys Ser Thr Val Cys Ala Asn Ile Ala Val Ala
    570                 575                 580

ttt gca caa caa ggt aaa aaa gta ctt tta att gat ggc gat ctt cgt    2060
Phe Ala Gln Gln Gly Lys Lys Val Leu Leu Ile Asp Gly Asp Leu Arg
585                 590                 595                 600

aaa ccg act gtt aac att act ttt aaa gta caa aat aga gta gga tta    2108
Lys Pro Thr Val Asn Ile Thr Phe Lys Val Gln Asn Arg Val Gly Leu
                605                 610                 615

acc aat att tta atg cat caa tct tcg att gaa gat gcc ata caa ggg    2156
Thr Asn Ile Leu Met His Gln Ser Ser Ile Glu Asp Ala Ile Gln Gly
            620                 625                 630

aca aga ctt tct gaa aat ctt aca ata att acc tct ggt cca att cca    2204
Thr Arg Leu Ser Glu Asn Leu Thr Ile Ile Thr Ser Gly Pro Ile Pro
        635                 640                 645

cct aat cca tcg gaa tta tta gca tct agt gca atg aag aat ttg att    2252
Pro Asn Pro Ser Glu Leu Leu Ala Ser Ser Ala Met Lys Asn Leu Ile
    650                 655                 660

gac tct gtg tcc gat tta ttt gat gtt gtt ttg att gat act cca act    2300
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Asp Ser Val Ser Asp Leu Phe Asp Val Val Leu Ile Asp Thr Pro Thr
665                 670                 675                 680

ctc tct gca gtt act gat gct caa att ttg agt agt tat gta gga gga    2348
Leu Ser Ala Val Thr Asp Ala Gln Ile Leu Ser Ser Tyr Val Gly Gly
                685                 690                 695

gca gtt att gtt gta cgt gcc tat gaa aca aaa aaa gag agt tta gca    2396
Ala Val Ile Val Val Arg Ala Tyr Glu Thr Lys Lys Glu Ser Leu Ala
            700                 705                 710

aaa aca aaa aaa atg ctt gaa caa gtt aat aca aat att tta ggg gtt    2444
Lys Thr Lys Lys Met Leu Glu Gln Val Asn Thr Asn Ile Leu Gly Val
        715                 720                 725

gtt ttg cat ggg gta aac tct tct gag tca cca tcg tat tac tac cac    2492
Val Leu His Gly Val Asn Ser Ser Glu Ser Pro Ser Tyr Tyr Tyr His
    730                 735                 740

gga gta gag taa ttggaataaa cttgaatcaa ataaaagaca gaaatttgta        2544
Gly Val Glu
745

gaagaggaga gcaaatgatt gatattcatt gccatatttt actggagcta aaacttctgg 2604

agatactttg aca atg ctg aaa tca gca att gat gaa ggg ata aca acc     2653
               Met Leu Lys Ser Ala Ile Asp Glu Gly Ile Thr Thr
                   750                 755                 760

atc act gcc act cct cat cat aat cct caa ttt aat aat gaa tca ccg    2701
Ile Thr Ala Thr Pro His His Asn Pro Gln Phe Asn Asn Glu Ser Pro
                765                 770                 775

ctt att ttg aag aaa gtt aag gaa gtt caa aat atc att gac gag cat    2749
Leu Ile Leu Lys Lys Val Lys Glu Val Gln Asn Ile Ile Asp Glu His
            780                 785                 790

caa tta cca att gaa gtt tta cca gga caa gag gtg aga ata tat ggt    2797
Gln Leu Pro Ile Glu Val Leu Pro Gly Gln Glu Val Arg Ile Tyr Gly
        795                 800                 805

gat tta tta aaa gaa ttt tct gaa gga aag tta ctg aca gca gcg ggc    2845
Asp Leu Leu Lys Glu Phe Ser Glu Gly Lys Leu Leu Thr Ala Ala Gly
    810                 815                 820

act tca agt tat ata ttg att gaa ttt cca tca aat cat gtg cca gct    2893
Thr Ser Ser Tyr Ile Leu Ile Glu Phe Pro Ser Asn His Val Pro Ala
825                 830                 835                 840

tat gct aaa gaa ctt ttt tat aat att caa ttg gag gga ctt caa cct    2941
Tyr Ala Lys Glu Leu Phe Tyr Asn Ile Gln Leu Glu Gly Leu Gln Pro
                845                 850                 855

att ttg gtc cac cct gag cgt aat agc gga atc att gag aac cct gat    2989
Ile Leu Val His Pro Glu Arg Asn Ser Gly Ile Ile Glu Asn Pro Asp
            860                 865                 870

ata tta ttt gat ttt att gaa caa gga gta cta agt cag ata aca gct    3037
Ile Leu Phe Asp Phe Ile Glu Gln Gly Val Leu Ser Gln Ile Thr Ala
        875                 880                 885
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tca agt gtc act ggt cat ttt ggt aaa aaa ata caa aag ctg tca ttt    3085
Ser Ser Val Thr Gly His Phe Gly Lys Lys Ile Gln Lys Leu Ser Phe
    890                 895                 900

aaa atg ata gaa aac cat ctt acg cat ttt gtt gca tca gat gcg cat    3133
Lys Met Ile Glu Asn His Leu Thr His Phe Val Ala Ser Asp Ala His
905                 910                 915                 920

aat gtg acg tca cgt gca ttt aag atg aag gaa gcg ttt gaa att att    3181
Asn Val Thr Ser Arg Ala Phe Lys Met Lys Glu Ala Phe Glu Ile Ile
                925                 930                 935

gaa gat agt tat ggt tct gat gta tca cga atg ttt caa aat aat gca    3229
Glu Asp Ser Tyr Gly Ser Asp Val Ser Arg Met Phe Gln Asn Asn Ala
            940                 945                 950

gag tca gtg att tta aac gaa agt ttt tat caa gaa aaa cca aca aag    3277
Glu Ser Val Ile Leu Asn Glu Ser Phe Tyr Gln Glu Lys Pro Thr Lys
        955                 960                 965

atc aaa aca aag aaa ttt tta gga tta ttt taa aaggattaaa aggagtaaat 3330
Ile Lys Thr Lys Lys Phe Leu Gly Leu Phe
    970                 975

a atg gaa ttt ttt gag gat gcc tca tca cct gaa tcg gga gag cct aag 3379
  Met Glu Phe Phe Glu Asp Ala Ser Ser Pro Glu Ser Gly Glu Pro Lys
  980                 985                 990                 995

tta gta gaa tta aaa aat ttt tct tat aga gag cta att ata aaa aga    3427
Leu Val Glu Leu Lys Asn Phe Ser Tyr Arg Glu Leu Ile Ile Lys Arg
               1000                1005                1010

gca att gat atc cta gga gga tta gca ggt tca gtt tta ttt ctt att    3475
Ala Ile Asp Ile Leu Gly Gly Leu Ala Gly Ser Val Leu Phe Leu Ile
           1015                1020                1025

gcg gct gca ttg ctt tat atc cct tac aaa atg agc tca aaa aaa gat    3523
Ala Ala Ala Leu Leu Tyr Ile Pro Tyr Lys Met Ser Ser Lys Lys Asp
       1030                1035                1040

caa ggg cca atg ttc tat aaa caa aaa cgc tat ggt aaa aat ggt aaa    3571
Gln Gly Pro Met Phe Tyr Lys Gln Lys Arg Tyr Gly Lys Asn Gly Lys
   1045                1050                1055

att ttt tat att ttg aaa ttt aga aca atg att ctt aat gcc gag cag    3619
Ile Phe Tyr Ile Leu Lys Phe Arg Thr Met Ile Leu Asn Ala Glu Gln
1060                1065                1070                1075

tat cta gaa ctt aat cca gat gtt aaa gct gct tac cat gcc aac ggc    3667
Tyr Leu Glu Leu Asn Pro Asp Val Lys Ala Ala Tyr His Ala Asn Gly
               1080                1085                1090

aat aag cta gaa aac gat cca cgg gta acg aag att ggc tca ttt ata    3715
Asn Lys Leu Glu Asn Asp Pro Arg Val Thr Lys Ile Gly Ser Phe Ile
           1095                1100                1105

aga cga cac tca att gat gaa ctg cca caa ttt atc aat gtt ctt aaa    3763
Arg Arg His Ser Ile Asp Glu Leu Pro Gln Phe Ile Asn Val Leu Lys
       1110                1115                1120

ggg gat atg tca tta gtt ggt cca aga cca att ctg ctt ttt gaa gcg    3811
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Gly Asp Met Ser Leu Val Gly Pro Arg Pro Ile Leu Leu Phe Glu Ala
1125 1130 1135

aaa gaa tat ggg aaa cgc ctc gct tac tta ctc atg tgc aaa cca gga 3859
Lys Glu Tyr Gly Lys Arg Leu Ala Tyr Leu Leu Met Cys Lys Pro Gly
1140 1145 1150 1155

atc act ggt tat tgg acg aca cat ggt cga agt aaa gtt ctt ttt cct 3907
Ile Thr Gly Tyr Trp Thr Thr His Gly Arg Ser Lys Val Leu Phe Pro
1160 1165 1170

caa cga gca gat tta gaa ctc tat tat ctc cag tac cat agc acc aaa 3955
Gln Arg Ala Asp Leu Glu Leu Tyr Tyr Leu Gln Tyr His Ser Thr Lys
1175 1180 1185

aat gat atc aag ctt cta gta ctc aca att gta caa agt att aac gga 4003
Asn Asp Ile Lys Leu Leu Val Leu Thr Ile Val Gln Ser Ile Asn Gly
1190 1195 1200

tcg gac gca tat taa aaa atg aaa ata gca tta gta ggt tcc agc ggt 4051
Ser Asp Ala Tyr Met Lys Ile Ala Leu Val Gly Ser Ser Gly
1205 1210 1215

ggc cat ttg aca cac ctg tat ttg tta aaa aag ttt tgg gaa aac gaa 4099
Gly His Leu Thr His Leu Tyr Leu Leu Lys Lys Phe Trp Glu Asn Glu
1220 1225 1230

gat aga ttt tgg gtc aca ttt gat aaa aca gat gca aaa tct ata ttg 4147
Asp Arg Phe Trp Val Thr Phe Asp Lys Thr Asp Ala Lys Ser Ile Leu
1235 1240 1245 1250

aaa gaa gaa aga ttt tat cct tgt tat tat ccc aca aat aga aat gta 4195
Lys Glu Glu Arg Phe Tyr Pro Cys Tyr Tyr Pro Thr Asn Arg Asn Val
1255 1260 1265

aaa aac acg ata aaa aat acc att ctt gca ttt aaa ata ctt aga aaa 4243
Lys Asn Thr Ile Lys Asn Thr Ile Leu Ala Phe Lys Ile Leu Arg Lys
1270 1275 1280

gaa aaa cca gat ttg att att tcg agt ggt gct gcg gta gcc gtt cct 4291
Glu Lys Pro Asp Leu Ile Ile Ser Ser Gly Ala Ala Val Ala Val Pro
1285 1290 1295

ttt ttt tgg tta ggt aaa cta ttc ggt gca aag aca gtc tat att gaa 4339
Phe Phe Trp Leu Gly Lys Leu Phe Gly Ala Lys Thr Val Tyr Ile Glu
1300 1305 1310

ata ttt gac cgg atc gat aaa cca acc tta aca gga aaa tta gtt tat 4387
Ile Phe Asp Arg Ile Asp Lys Pro Thr Leu Thr Gly Lys Leu Val Tyr
1315 1320 1325 1330

cca gtt act gat aag ttt ata gtt caa tgg gaa gag tta aaa aaa gtt 4435
Pro Val Thr Asp Lys Phe Ile Val Gln Trp Glu Glu Leu Lys Lys Val
1335 1340 1345

tac cct aaa gca att aat tta gga gga att ttc taa tgattttgt 4481
Tyr Pro Lys Ala Ile Asn Leu Gly Gly Ile Phe
1350 1355

aacggttgga actcacgaac aaccatttaa tcgactcatt caaaaaattg atgaacttgt 4541

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acgcgatggt gaaatcgaag acgatgtatt catgcaaatt gggtactcaa cttatgaacc 4601

taaatatact aaatgggaaa agtttattgg atatgagact atggaaagat gtatgaatga 4661

agcgagtacg attattactc atggcggacc atctacctat atgcaagtat tacaactagg 4721

taaaattccg atagttgttc cacggcaaat gaaatttgat gagcatataa atgatcatca 4781

actttgggta agtaaacagg ttgtgaaaaa gggatactca ttgattttgt gcgaagatgt 4841

tgaagacatt ctcgaaaata ttattagttc caaaatttca gataccttac aaaaaaatgt 4901

aaatcacaac actgaattca taaaattatt cagtgctgaa atttaccagc tatttataaa 4961

aagtgagaag at atg ata cca aaa gta ata cac tat tgc tgg ttc gga ggg 5012
              Met Ile Pro Lys Val Ile His Tyr Cys Trp Phe Gly Gly
                  1360                1365                1370

caa cct tta cca gaa tct gcg cta aaa tgt att gaa agt tgg aga agg   5060
Gln Pro Leu Pro Glu Ser Ala Leu Lys Cys Ile Glu Ser Trp Arg Arg
            1375                1380                1385

ttt tgt cca gat tat gaa ata aaa caa tgg tct gag aaa aac tat gat   5108
Phe Cys Pro Asp Tyr Glu Ile Lys Gln Trp Ser Glu Lys Asn Tyr Asp
        1390                1395                1400

gta aat aaa att caa tat att aag gaa gca tat caa gaa aaa aaa ttt   5156
Val Asn Lys Ile Gln Tyr Ile Lys Glu Ala Tyr Gln Glu Lys Lys Phe
    1405                1410                1415

gct ttt gtc acg gat gtt gca agg ctc gat ata att tgg aat gaa ggc   5204
Ala Phe Val Thr Asp Val Ala Arg Leu Asp Ile Ile Trp Asn Glu Gly
1420                1425                1430                1435

ggt ata tat ctt gac acg gat gta gag ctt ata aaa tct ctt gat gaa   5252
Gly Ile Tyr Leu Asp Thr Asp Val Glu Leu Ile Lys Ser Leu Asp Glu
                1440                1445                1450

ttg ctg tat aat agt tta tat tta gga atg gaa aga gct ggt aga gta   5300
Leu Leu Tyr Asn Ser Leu Tyr Leu Gly Met Glu Arg Ala Gly Arg Val
            1455                1460                1465

aat acg ggt tta ggg ttt gga gct gaa gta aat cat cca att gtg aga   5348
Asn Thr Gly Leu Gly Phe Gly Ala Glu Val Asn His Pro Ile Val Arg
        1470                1475                1480

gct aat tta gaa ttg tat act aat att cct ttt tca ggc aat gat aat   5396
Ala Asn Leu Glu Leu Tyr Thr Asn Ile Pro Phe Ser Gly Asn Asp Asn
    1485                1490                1495

ata act tgt gtg acc tat acg acg aat ctt ttg aaa aaa tat ggt cta   5444
Ile Thr Cys Val Thr Tyr Thr Thr Asn Leu Leu Lys Lys Tyr Gly Leu
1500                1505                1510                1515

aaa aac aac aat gaa att caa cat ata gat aac gca ata att tta cct   5492
Lys Asn Asn Asn Glu Ile Gln His Ile Asp Asn Ala Ile Ile Leu Pro
                1520                1525                1530

act gaa tat tta tgt cct cta agt ttt gaa aca aat cga tta aaa ata   5540
Thr Glu Tyr Leu Cys Pro Leu Ser Phe Glu Thr Asn Arg Leu Lys Ile
            1535                1540                1545
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acg gaa aat act tac tcc atc cat cac tat gat atg agt tgg aaa gat    5588
Thr Glu Asn Thr Tyr Ser Ile His His Tyr Asp Met Ser Trp Lys Asp
      1550                1555                1560

aag aga gat aaa ttt tta aga ctt aaa ata caa ctt aga aaa tgg gta    5636
Lys Arg Asp Lys Phe Leu Arg Leu Lys Ile Gln Leu Arg Lys Trp Val
  1565                1570                1575

ggt gat gat ttt tat gaa aaa gtt att aaa aga att gga aaa taa ttatc 5686
Gly Asp Asp Phe Tyr Glu Lys Val Ile Lys Arg Ile Gly Lys
1580                1585                1590

atg aat aaa ata acc atg aca aga gag atg aga gtt att gcc tta tgt    5734
Met Asn Lys Ile Thr Met Thr Arg Glu Met Arg Val Ile Ala Leu Cys
1595                1600                1605                1610

gtc gta att tta gaa tat tta aat aat aca gga tta att gcg tct tca    5782
Val Val Ile Leu Glu Tyr Leu Asn Asn Thr Gly Leu Ile Ala Ser Ser
                1615                1620                1625

gca tac tct ttt agc atg gcg agt aca atc ctc tta tcc tat atc tta    5830
Ala Tyr Ser Phe Ser Met Ala Ser Thr Ile Leu Leu Ser Tyr Ile Leu
            1630                1635                1640

ttc tgt aaa aaa aga aaa gga ttt tct tta aag gag att att gta cta    5878
Phe Cys Lys Lys Arg Lys Gly Phe Ser Leu Lys Glu Ile Ile Val Leu
      1645                1650                1655

cta att cca ttt att ttt gta gtt tta aat cgt gat cct agt aat ttc    5926
Leu Ile Pro Phe Ile Phe Val Val Leu Asn Arg Asp Pro Ser Asn Phe
  1660                1665                1670

agt tta ggg tta atg tgg ata ctc tat ttt atg tta agt aag tcg gaa    5974
Ser Leu Gly Leu Met Trp Ile Leu Tyr Phe Met Leu Ser Lys Ser Glu
1675                1680                1685                1690

ata gat tta aaa aaa gtg atg aaa aca ttt ttt gtt acc tct agt gtt    6022
Ile Asp Leu Lys Lys Val Met Lys Thr Phe Phe Val Thr Ser Ser Val
                1695                1700                1705

tgt ttt att ttg aca ata gta ctt tat tta ata atg tct ctt aat aaa    6070
Cys Phe Ile Leu Thr Ile Val Leu Tyr Leu Ile Met Ser Leu Asn Lys
            1710                1715                1720

agc tct gat atg ata atg tgg cgt gga gat gct ttt ata aat cgt atg    6118
Ser Ser Asp Met Ile Met Trp Arg Gly Asp Ala Phe Ile Asn Arg Met
      1725                1730                1735

agt tta gga ttt atc caa ccg aat ttt gca atg atg agc ttt tta ggt    6166
Ser Leu Gly Phe Ile Gln Pro Asn Phe Ala Met Met Ser Phe Leu Gly
  1740                1745                1750

ata gcg ata gcc tta tta tat ttg agt act gaa aga caa aga ata act    6214
Ile Ala Ile Ala Leu Leu Tyr Leu Ser Thr Glu Arg Gln Arg Ile Thr
1755                1760                1765                1770

ata att ttt att gcc att gta act ttt att ata ttt tac ttt act caa    6262
Ile Ile Phe Ile Ala Ile Val Thr Phe Ile Ile Phe Tyr Phe Thr Gln
                1775                1780                1785
```

tca aga act tca gga tat atc tta ttt ttt att ttg agt att tta ttt 6310
Ser Arg Thr Ser Gly Tyr Ile Leu Phe Phe Ile Leu Ser Ile Leu Phe
1790 1795 1800

gtt agt agt aaa aaa act aaa aag caa gtt tca aat ttt gaa aaa agg 6358
Val Ser Ser Lys Lys Thr Lys Lys Gln Val Ser Asn Phe Glu Lys Arg
1805 1810 1815

agc att aca gtt tta cca cta ctt ctt tta atc atc tct tat tcg ttg 6406
Ser Ile Thr Val Leu Pro Leu Leu Leu Leu Ile Ile Ser Tyr Ser Leu
1820 1825 1830

tta aag tta cct att aat caa tac atc aat agc ttg ctt tct ggt cgt 6454
Leu Lys Leu Pro Ile Asn Gln Tyr Ile Asn Ser Leu Leu Ser Gly Arg
1835 1840 1845 1850

ctg gcg ctt tat caa gag att tat tct aca ttt ggt ata cat ttg ata 6502
Leu Ala Leu Tyr Gln Glu Ile Tyr Ser Thr Phe Gly Ile His Leu Ile
1855 1860 1865

ggg aat aat gat gtt aaa aat aca atg tta gat aca gca tat ctt caa 6550
Gly Asn Asn Asp Val Lys Asn Thr Met Leu Asp Thr Ala Tyr Leu Gln
1870 1875 1880

agt ttg cta gca aaa gga att ttg ttt aca ttg ttt tta ttt gta act 6598
Ser Leu Leu Ala Lys Gly Ile Leu Phe Thr Leu Phe Leu Phe Val Thr
1885 1890 1895

ttc ttt ttc ata ttt ttt ctt aag aga aaa aca caa act agg ttg caa 6646
Phe Phe Phe Ile Phe Phe Leu Lys Arg Lys Thr Gln Thr Arg Leu Gln
1900 1905 1910

agt tta gta att atg atg tat ttt tta att gca ttt aca gaa aca tca 6694
Ser Leu Val Ile Met Met Tyr Phe Leu Ile Ala Phe Thr Glu Thr Ser
1915 1920 1925 1930

ttt ttt agg ttt gta att tta ttt cca gta ttg atg gta ata atg gat 6742
Phe Phe Arg Phe Val Ile Leu Phe Pro Val Leu Met Val Ile Met Asp
1935 1940 1945

cag aaa gag gct aat aaa gta ata gaa aag gtg gca tag tgagtattaa 6791
Gln Lys Glu Ala Asn Lys Val Ile Glu Lys Val Ala
1950 1955

taaaacagag attgaggaat acaaagtatc cgttatagtt cctgtttaca atgtagagg 6850

<210> 2
<211> 105
<212> PRT
<213> Lactococcus lactis

<400> 2
Met Asn Asn Leu Phe Tyr His Arg Leu Lys Glu Leu Val Glu Ser Ser
1 5 10 15
Gly Lys Ser Ala Asn Gln Ile Glu Arg Glu Leu Gly Tyr Pro Arg Asn
20 25 30
Ser Leu Asn Asn Tyr Lys Leu Gly Gly Glu Pro Ser Gly Thr Arg Leu
35 40 45
Ile Gly Leu Ser Glu Tyr Phe Asn Val Ser Pro Lys Tyr Leu Met Gly
50 55 60

```
Ile Ile Asp Glu Pro Asn Asp Ser Ser Ala Ile Asn Leu Phe Lys Thr
65                  70                  75                  80
Leu Thr Gln Glu Glu Lys Lys Glu Met Phe Ile Ile Cys Gln Lys Trp
                85                  90                  95
Leu Phe Leu Glu Tyr Gln Ile Glu Leu
            100                 105


<210> 3
<211> 150
<212> PRT
<213> Lactococcus lactis

<400> 3
Asn Lys Phe Trp Asn Ile Lys Asn Ile Thr Tyr Asn Gly Glu Thr Ser
 1               5                  10                  15
Glu Gln Leu Leu Ala Glu Lys Val Gln Asn Gln Val Leu Ala Thr Asn
            20                  25                  30
Pro Asp Val Val Leu Tyr Glu Ala Pro Leu Phe Asn Asp Asn Gln Asn
        35                  40                  45
Ile Glu Ala Thr Ala Ser Trp Thr Ser Asn Glu Gln Leu Ile Thr Asn
    50                  55                  60
Leu Ala Ser Thr Gly Ala Glu Val Ile Val Gln Pro Ser Pro Pro Ile
65                  70                  75                  80
Tyr Gly Gly Val Val Tyr Pro Val Gln Glu Glu Gln Phe Lys Gln Ser
                85                  90                  95
Leu Ser Thr Lys Tyr Pro Tyr Ile Asp Tyr Trp Ala Ser Tyr Pro Asp
            100                 105                 110
Lys Asn Ser Asp Glu Met Lys Gly Leu Val Ser Asp Asp Gly Val Tyr
        115                 120                 125
Arg Thr Leu Asn Ala Ser Gly Asn Lys Val Trp Leu Asp Tyr Ile Thr
    130                 135                 140
Lys Tyr Phe Thr Ala Asn
145                 150


<210> 4
<211> 259
<212> PRT
<213> Lactococcus lactis

<400> 4
Met Gln Glu Thr Gln Glu Gln Thr Ile Asp Leu Arg Gly Ile Phe Lys
 1               5                  10                  15
Ile Ile Arg Lys Arg Leu Gly Leu Ile Leu Phe Ser Ala Leu Ile Val
            20                  25                  30
Thr Ile Leu Gly Ser Ile Tyr Thr Phe Phe Ile Ala Ser Pro Val Tyr
        35                  40                  45
Thr Ala Ser Thr Gln Leu Val Val Lys Leu Pro Asn Ser Glu His Ser
    50                  55                  60
Ala Ala Tyr Ala Gly Glu Val Thr Gly Asn Ile Gln Met Ala Asn Thr
65                  70                  75                  80
Ile Asn Gln Val Ile Val Ser Pro Val Ile Leu Asp Lys Val Gln Ser
                85                  90                  95
Asn Leu Asn Leu Ser Asp Gly Ser Phe Gln Lys Gln Val Thr Val Ala
            100                 105                 110
Asn Gln Thr Asp Ser Gln Val Ile Thr Leu Thr Val Lys Tyr Ser Asn
        115                 120                 125
Pro Tyr Ile Ala Gln Lys Ile Ala Asp Glu Thr Ala Lys Ile Phe Ser
    130                 135                 140
Ser Asp Ala Ala Lys Leu Leu Asn Val Thr Asn Val Asn Ile Leu Ser
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145 150 155 160
Lys Ala Lys Ala Gln Thr Thr Pro Ile Ser Pro Lys Pro Lys Leu Tyr
165 170 175
Leu Ala Ile Ser Val Ile Ala Gly Leu Val Leu Gly Leu Ala Ile Ala
180 185 190
Leu Leu Lys Glu Leu Phe Asp Asn Lys Ile Asn Lys Glu Glu Asp Ile
195 200 205
Glu Ala Leu Gly Leu Thr Val Leu Gly Val Thr Ser Tyr Ala Gln Met
210 215 220
Ser Asp Phe Asn Lys Asn Thr Asn Lys Asn Gly Thr Gln Ser Gly Thr
225 230 235 240
Lys Ser Ser Pro Pro Ser Asp His Glu Val Asn Arg Ser Ser Lys Arg
245 250 255
Asn Lys Arg

<210> 5
<211> 231
<212> PRT
<213> Lactococcus lactis

<400> 5
Met Ala Lys Asn Lys Arg Ser Ile Asp Asn Asn Arg Tyr Ile Ile Thr
1 5 10 15
Ser Val Asn Pro Gln Ser Pro Ile Ser Glu Gln Tyr Arg Ser Ile Arg
20 25 30
Thr Thr Ile Asp Phe Lys Met Ala Asp Gln Gly Ile Lys Ser Phe Leu
35 40 45
Val Ala Ser Ser Glu Val Ala Val Gly Lys Ser Thr Val Cys Ala Asn
50 55 60
Ile Ala Val Ala Phe Ala Gln Gln Gly Lys Lys Val Leu Leu Ile Asp
65 70 75 80
Gly Asp Leu Arg Lys Pro Thr Val Asn Ile Thr Phe Lys Val Gln Asn
85 90 95
Arg Val Gly Leu Thr Asn Ile Leu Met His Gln Ser Ser Ile Glu Asp
100 105 110
Ala Ile Gln Gly Thr Arg Leu Ser Glu Asn Leu Thr Ile Ile Thr Ser
115 120 125
Gly Pro Ile Pro Pro Asn Pro Ser Glu Leu Leu Ala Ser Ser Ala Met
130 135 140
Lys Asn Leu Ile Asp Ser Val Ser Asp Leu Phe Asp Val Val Leu Ile
145 150 155 160
Asp Thr Pro Thr Leu Ser Ala Val Thr Asp Ala Gln Ile Leu Ser Ser
165 170 175
Tyr Val Gly Gly Ala Val Ile Val Val Arg Ala Tyr Glu Thr Lys Lys
180 185 190
Glu Ser Leu Ala Lys Thr Lys Lys Met Leu Glu Gln Val Asn Thr Asn
195 200 205
Ile Leu Gly Val Val Leu His Gly Val Asn Ser Ser Glu Ser Pro Ser
210 215 220
Tyr Tyr Tyr His Gly Val Glu
225 230

<210> 6
<211> 230
<212> PRT
<213> Lactococcus lactis

<400> 6
Met Leu Lys Ser Ala Ile Asp Glu Gly Ile Thr Thr Ile Thr Ala Thr

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  1               5                  10                  15
Pro His His Asn Pro Gln Phe Asn Asn Glu Ser Pro Leu Ile Leu Lys
            20                  25                  30
Lys Val Lys Glu Val Gln Asn Ile Ile Asp Glu His Gln Leu Pro Ile
        35                  40                  45
Glu Val Leu Pro Gly Gln Glu Val Arg Ile Tyr Gly Asp Leu Leu Lys
    50                  55                  60
Glu Phe Ser Glu Gly Lys Leu Leu Thr Ala Ala Gly Thr Ser Ser Tyr
65                  70                  75                      80
Ile Leu Ile Glu Phe Pro Ser Asn His Val Pro Ala Tyr Ala Lys Glu
                85                  90                  95
Leu Phe Tyr Asn Ile Gln Leu Glu Gly Leu Gln Pro Ile Leu Val His
            100                 105                 110
Pro Glu Arg Asn Ser Gly Ile Ile Glu Asn Pro Asp Ile Leu Phe Asp
        115                 120                 125
Phe Ile Glu Gln Gly Val Leu Ser Gln Ile Thr Ala Ser Ser Val Thr
    130                 135                 140
Gly His Phe Gly Lys Lys Ile Gln Lys Leu Ser Phe Lys Met Ile Glu
145                 150                 155                     160
Asn His Leu Thr His Phe Val Ala Ser Asp Ala His Asn Val Thr Ser
                165                 170                 175
Arg Ala Phe Lys Met Lys Glu Ala Phe Glu Ile Ile Glu Asp Ser Tyr
            180                 185                 190
Gly Ser Asp Val Ser Arg Met Phe Gln Asn Asn Ala Glu Ser Val Ile
        195                 200                 205
Leu Asn Glu Ser Phe Tyr Gln Glu Lys Pro Thr Lys Ile Lys Thr Lys
    210                 215                 220
Lys Phe Leu Gly Leu Phe
225                 230


<210> 7
<211> 228
<212> PRT
<213> Lactococcus lactis

<400> 7
Met Glu Phe Phe Glu Asp Ala Ser Ser Pro Glu Ser Gly Glu Pro Lys
  1               5                  10                  15
Leu Val Glu Leu Lys Asn Phe Ser Tyr Arg Glu Leu Ile Ile Lys Arg
            20                  25                  30
Ala Ile Asp Ile Leu Gly Gly Leu Ala Gly Ser Val Leu Phe Leu Ile
        35                  40                  45
Ala Ala Ala Leu Leu Tyr Ile Pro Tyr Lys Met Ser Ser Lys Lys Asp
    50                  55                  60
Gln Gly Pro Met Phe Tyr Lys Gln Lys Arg Tyr Gly Lys Asn Gly Lys
65                  70                  75                      80
Ile Phe Tyr Ile Leu Lys Phe Arg Thr Met Ile Leu Asn Ala Glu Gln
                85                  90                  95
Tyr Leu Glu Leu Asn Pro Asp Val Lys Ala Ala Tyr His Ala Asn Gly
            100                 105                 110
Asn Lys Leu Glu Asn Asp Pro Arg Val Thr Lys Ile Gly Ser Phe Ile
        115                 120                 125
Arg Arg His Ser Ile Asp Glu Leu Pro Gln Phe Ile Asn Val Leu Lys
    130                 135                 140
Gly Asp Met Ser Leu Val Gly Pro Arg Pro Ile Leu Leu Phe Glu Ala
145                 150                 155                     160
Lys Glu Tyr Gly Lys Arg Leu Ala Tyr Leu Leu Met Cys Lys Pro Gly
                165                 170                 175
Ile Thr Gly Tyr Trp Thr Thr His Gly Arg Ser Lys Val Leu Phe Pro
            180                 185                 190
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```
Gln Arg Ala Asp Leu Glu Leu Tyr Tyr Leu Gln Tyr His Ser Thr Lys
        195                 200                 205
Asn Asp Ile Lys Leu Leu Val Leu Thr Ile Val Gln Ser Ile Asn Gly
    210                 215                 220
Ser Asp Ala Tyr
225
```

<210> 8
<211> 149
<212> PRT
<213> Lactococcus lactis

<400> 8

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Met Lys Ile Ala Leu Val Gly Ser Ser Gly Gly His Leu Thr His Leu
  1               5                  10                  15
Tyr Leu Leu Lys Lys Phe Trp Glu Asn Glu Asp Arg Phe Trp Val Thr
             20                  25                  30
Phe Asp Lys Thr Asp Ala Lys Ser Ile Leu Lys Glu Glu Arg Phe Tyr
         35                  40                  45
Pro Cys Tyr Tyr Pro Thr Asn Arg Asn Val Lys Asn Thr Ile Lys Asn
     50                  55                  60
Thr Ile Leu Ala Phe Lys Ile Leu Arg Lys Glu Lys Pro Asp Leu Ile
 65                  70                  75                  80
Ile Ser Ser Gly Ala Ala Val Ala Val Pro Phe Phe Trp Leu Gly Lys
                 85                  90                  95
Leu Phe Gly Ala Lys Thr Val Tyr Ile Glu Ile Phe Asp Arg Ile Asp
            100                 105                 110
Lys Pro Thr Leu Thr Gly Lys Leu Val Tyr Pro Val Thr Asp Lys Phe
        115                 120                 125
Ile Val Gln Trp Glu Glu Leu Lys Lys Val Tyr Pro Lys Ala Ile Asn
    130                 135                 140
Leu Gly Gly Ile Phe
145
```

<210> 9
<211> 235
<212> PRT
<213> Lactococcus lactis

<400> 9

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Met Ile Pro Lys Val Ile His Tyr Cys Trp Phe Gly Gly Gln Pro Leu
  1               5                  10                  15
Pro Glu Ser Ala Leu Lys Cys Ile Glu Ser Trp Arg Arg Phe Cys Pro
             20                  25                  30
Asp Tyr Glu Ile Lys Gln Trp Ser Glu Lys Asn Tyr Asp Val Asn Lys
         35                  40                  45
Ile Gln Tyr Ile Lys Glu Ala Tyr Gln Glu Lys Lys Phe Ala Phe Val
     50                  55                  60
Thr Asp Val Ala Arg Leu Asp Ile Ile Trp Asn Glu Gly Gly Ile Tyr
 65                  70                  75                  80
Leu Asp Thr Asp Val Glu Leu Ile Lys Ser Leu Asp Glu Leu Leu Tyr
                 85                  90                  95
Asn Ser Leu Tyr Leu Gly Met Glu Arg Ala Gly Arg Val Asn Thr Gly
            100                 105                 110
Leu Gly Phe Gly Ala Glu Val Asn His Pro Ile Val Arg Ala Asn Leu
        115                 120                 125
Glu Leu Tyr Thr Asn Ile Pro Phe Ser Gly Asn Asp Asn Ile Thr Cys
    130                 135                 140
Val Thr Tyr Thr Thr Asn Leu Leu Lys Lys Tyr Gly Leu Lys Asn Asn
```

```
145                 150                 155                 160
Asn Glu Ile Gln His Ile Asp Asn Ala Ile Ile Leu Pro Thr Glu Tyr
                165                 170                 175
Leu Cys Pro Leu Ser Phe Glu Thr Asn Arg Leu Lys Ile Thr Glu Asn
            180                 185                 190
Thr Tyr Ser Ile His His Tyr Asp Met Ser Trp Lys Asp Lys Arg Asp
        195                 200                 205
Lys Phe Leu Arg Leu Lys Ile Gln Leu Arg Lys Trp Val Gly Asp Asp
    210                 215                 220
Phe Tyr Glu Lys Val Ile Lys Arg Ile Gly Lys
225                 230                 235
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<210> 10
<211> 364
<212> PRT
<213> Lactococcus lactis

<400> 10

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Met Asn Lys Ile Thr Met Thr Arg Glu Met Arg Val Ile Ala Leu Cys
  1               5                  10                  15
Val Val Ile Leu Glu Tyr Leu Asn Asn Thr Gly Leu Ile Ala Ser Ser
            20                  25                  30
Ala Tyr Ser Phe Ser Met Ala Ser Thr Ile Leu Leu Ser Tyr Ile Leu
        35                  40                  45
Phe Cys Lys Lys Arg Lys Gly Phe Ser Leu Lys Glu Ile Ile Val Leu
    50                  55                  60
Leu Ile Pro Phe Ile Phe Val Val Leu Asn Arg Asp Pro Ser Asn Phe
65                  70                  75                  80
Ser Leu Gly Leu Met Trp Ile Leu Tyr Phe Met Leu Ser Lys Ser Glu
                85                  90                  95
Ile Asp Leu Lys Lys Val Met Lys Thr Phe Phe Val Thr Ser Ser Val
            100                 105                 110
Cys Phe Ile Leu Thr Ile Val Leu Tyr Leu Ile Met Ser Leu Asn Lys
        115                 120                 125
Ser Ser Asp Met Ile Met Trp Arg Gly Asp Ala Phe Ile Asn Arg Met
    130                 135                 140
Ser Leu Gly Phe Ile Gln Pro Asn Phe Ala Met Met Ser Phe Leu Gly
145                 150                 155                 160
Ile Ala Ile Ala Leu Leu Tyr Leu Ser Thr Glu Arg Gln Arg Ile Thr
                165                 170                 175
Ile Ile Phe Ile Ala Ile Val Thr Phe Ile Ile Phe Tyr Phe Thr Gln
            180                 185                 190
Ser Arg Thr Ser Gly Tyr Ile Leu Phe Phe Ile Leu Ser Ile Leu Phe
        195                 200                 205
Val Ser Ser Lys Lys Thr Lys Lys Gln Val Ser Asn Phe Glu Lys Arg
    210                 215                 220
Ser Ile Thr Val Leu Pro Leu Leu Leu Leu Ile Ile Ser Tyr Ser Leu
225                 230                 235                 240
Leu Lys Leu Pro Ile Asn Gln Tyr Ile Asn Ser Leu Leu Ser Gly Arg
                245                 250                 255
Leu Ala Leu Tyr Gln Glu Ile Tyr Ser Thr Phe Gly Ile His Leu Ile
            260                 265                 270
Gly Asn Asn Asp Val Lys Asn Thr Met Leu Asp Thr Ala Tyr Leu Gln
        275                 280                 285
Ser Leu Leu Ala Lys Gly Ile Leu Phe Thr Leu Phe Leu Phe Val Thr
    290                 295                 300
Phe Phe Phe Ile Phe Phe Leu Lys Arg Lys Thr Gln Thr Arg Leu Gln
305                 310                 315                 320
Ser Leu Val Ile Met Met Tyr Phe Leu Ile Ala Phe Thr Glu Thr Ser
                325                 330                 335
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```
Phe Phe Arg Phe Val Ile Leu Phe Pro Val Leu Met Val Ile Met Asp
            340                 345                 350
Gln Lys Glu Ala Asn Lys Val Ile Glu Lys Val Ala
        355                 360



<210> 11
<211> 168
<212> PRT
<213> Lactococcus lactis

<400> 11
Met Ile Phe Val Thr Val Gly Thr His Glu Gln Pro Phe Asn Arg Leu
  1               5                  10                  15
Ile Gln Lys Ile Asp Glu Leu Val Arg Asp Gly Glu Ile Glu Asp Asp
            20                  25                  30
Val Phe Met Gln Ile Gly Tyr Ser Thr Tyr Glu Pro Lys Tyr Thr Lys
        35                  40                  45
Trp Glu Lys Phe Ile Gly Tyr Glu Thr Met Glu Arg Cys Met Asn Glu
    50                  55                  60
Ala Ser Thr Ile Ile Thr His Gly Gly Pro Ser Thr Tyr Met Gln Val
 65                  70                  75                  80
Leu Gln Leu Gly Lys Ile Pro Ile Val Val Pro Arg Gln Met Lys Phe
                 85                  90                  95
Asp Glu His Ile Asn Asp His Gln Leu Trp Val Ser Lys Gln Val Val
            100                 105                 110
Lys Lys Gly Tyr Ser Leu Ile Leu Cys Glu Asp Val Glu Asp Ile Leu
        115                 120                 125
Glu Asn Ile Ile Ser Ser Lys Ile Ser Asp Thr Leu Gln Lys Asn Val
    130                 135                 140
Asn His Asn Thr Glu Phe Ile Lys Leu Phe Ser Ala Glu Ile Tyr Gln
145                 150                 155                 160
Leu Phe Ile Lys Ser Glu Lys Ile
                165


<210>  12
<211>  2349
<212>  DNA
<213>  Lactoccocus Lactis

<220>
<221>  CDS
<222>  (61)..(1056)

<220>
<221>  CDS
<222>  (1336)..(2322)

<400>  12
cagagagaaa attatttaaa aagggaactt aattaagctt aaaattgggg gagtataaaa      60

ttg agc gaa aat tta atc agt att ata gta cca gtt tat aat tca gaa       108
Leu Ser Glu Asn Leu Ile Ser Ile Ile Val Pro Val Tyr Asn Ser Glu
1               5                   10                  15

aag tat tta aga gcg gct att cat agt cta tta aat caa act tat caa       156
Lys Tyr Leu Arg Ala Ala Ile His Ser Leu Leu Asn Gln Thr Tyr Gln
            20                  25                  30
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aat att gaa gtt att ttg att aat gat ggg tcc act gat ggc tca caa      204
Asn Ile Glu Val Ile Leu Ile Asn Asp Gly Ser Thr Asp Gly Ser Gln
        35                  40                  45

gag cta att agc tca ttt caa aaa aag gat aaa aga att aaa tta tat      252
Glu Leu Ile Ser Ser Phe Gln Lys Lys Asp Lys Arg Ile Lys Leu Tyr
    50                  55                  60

aat act aaa aat ctg ggg gta tcg cat gcg aga aat tat ggt att gat      300
Asn Thr Lys Asn Leu Gly Val Ser His Ala Arg Asn Tyr Gly Ile Asp
65                  70                  75                  80

aga gct agt ggt tcg tat att atg ttt tta gac cca gac gac act tat      348
Arg Ala Ser Gly Ser Tyr Ile Met Phe Leu Asp Pro Asp Asp Thr Tyr
                85                  90                  95

gat aaa agt tac tgt tta gaa atg att ggg ttg att aat aag ttt aat      396
Asp Lys Ser Tyr Cys Leu Glu Met Ile Gly Leu Ile Asn Lys Phe Asn
            100                 105                 110

gct gat gtt gtt atg agt aat tac tat ata tgc aaa ggc aaa aat ata      444
Ala Asp Val Val Met Ser Asn Tyr Tyr Ile Cys Lys Gly Lys Asn Ile
        115                 120                 125

tat cct aat gtt aat aat gat ctt ctt gaa tgt gaa ggc ctc cta tca      492
Tyr Pro Asn Val Asn Asn Asp Leu Leu Glu Cys Glu Gly Leu Leu Ser
    130                 135                 140

agg gat aaa aca atg cgt tca ata cta tct gat aca ggt ttt aaa ggg      540
Arg Asp Lys Thr Met Arg Ser Ile Leu Ser Asp Thr Gly Phe Lys Gly
145                 150                 155                 160

ttt gta tgg aca aga att ttt aga aaa aat gta att aat aat gtt aaa      588
Phe Val Trp Thr Arg Ile Phe Arg Lys Asn Val Ile Asn Asn Val Lys
                165                 170                 175

ttc aat gag agc ata aat tac tta gaa gac atg tta ttt aat att agt      636
Phe Asn Glu Ser Ile Asn Tyr Leu Glu Asp Met Leu Phe Asn Ile Ser
            180                 185                 190

att gta cat aat gca aga att ata gcc tat aca aat aaa aga cat tat      684
Ile Val His Asn Ala Arg Ile Ile Ala Tyr Thr Asn Lys Arg His Tyr
        195                 200                 205

ttt tat tta caa aga gaa gat tct gca tca aaa aaa ttt agc aaa tct      732
Phe Tyr Leu Gln Arg Glu Asp Ser Ala Ser Lys Lys Phe Ser Lys Ser
    210                 215                 220

ttt ttt aaa tcc ctt aat ctt att aga ggg aaa gtt gat cct gaa ttt      780
Phe Phe Lys Ser Leu Asn Leu Ile Arg Gly Lys Val Asp Pro Glu Phe
225                 230                 235                 240

tat tcg caa att gat tct gtt att ttt tat aat tta gtt gga tgg tta      828
Tyr Ser Gln Ile Asp Ser Val Ile Phe Tyr Asn Leu Val Gly Trp Leu
                245                 250                 255

ata act gag aga aag agt agg gaa aat agt caa ttt ata agg aga aat      876
Ile Thr Glu Arg Lys Ser Arg Glu Asn Ser Gln Phe Ile Arg Arg Asn
            260                 265                 270

att aaa aat atg aaa tcc caa gtt aag ttt aaa acg ctt aaa atg gaa      924
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Ile Lys Asn Met Lys Ser Gln Val Lys Phe Lys Thr Leu Lys Met Glu
275 280 285

aac cca ata aaa aat tta ata tta aaa tta agc tat gct ttt ccc tta 972
Asn Pro Ile Lys Asn Leu Ile Leu Lys Leu Ser Tyr Ala Phe Pro Leu
290 295 300

gta gga tcg tgt atg ata cat atg tta tcc gtt ttt atg aaa acc aaa 1020
Val Gly Ser Cys Met Ile His Met Leu Ser Val Phe Met Lys Thr Lys
305 310 315 320

ctt tat tcc aaa tta atg agt atg tta agg aaa ggg tgaatcaaaa 1066
Leu Tyr Ser Lys Leu Met Ser Met Leu Arg Lys Gly
325 330

acaatattta agataaattt tggggttaaa accaattctg tgggttggac atacattaaa 1126

tctaaagcat ttttaatgcg agtcttgacc gtggtcatag gggatttgac ttctaagaat 1186

gttgttaagc attactaacg gagttagaat tttagagagc gtaaaatatc ttgtgataat 1246

tattaactta tcaagtacag accaaaatac tggagtttaa caggaactgt tagaatataa 1306

ttttatataa ttaggagtag aataaagag atg aat cca tta ata tca att att 1359
Met Asn Pro Leu Ile Ser Ile Ile
335 340

gtt cca ata tac aat gtt gag aag tat att ggt agt tta gta aat tct 1407
Val Pro Ile Tyr Asn Val Glu Lys Tyr Ile Gly Ser Leu Val Asn Ser
345 350 355

cta ttg aaa caa acg aac aag aat ttt gag gtt att ttt att gat gac 1455
Leu Leu Lys Gln Thr Asn Lys Asn Phe Glu Val Ile Phe Ile Asp Asp
360 365 370

gga tca act gat gaa agc atg caa att ttg aaa gaa ata atg gca ggc 1503
Gly Ser Thr Asp Glu Ser Met Gln Ile Leu Lys Glu Ile Met Ala Gly
375 380 385

agt gaa caa gaa ttt tcg ttc aag ttg ttg caa caa gtt aat cag ggt 1551
Ser Glu Gln Glu Phe Ser Phe Lys Leu Leu Gln Gln Val Asn Gln Gly
390 395 400

tta tct tca gcc agg aat atc ggt ata ctt aat gca act gga gaa tat 1599
Leu Ser Ser Ala Arg Asn Ile Gly Ile Leu Asn Ala Thr Gly Glu Tyr
405 410 415 420

atc ttt ttt ttg gat tca gat gat gaa ata gaa agc aat ttt gtg gag 1647
Ile Phe Phe Leu Asp Ser Asp Asp Glu Ile Glu Ser Asn Phe Val Glu
425 430 435

aca att ttg act agt tgc tat aaa tac agt caa ccg gat aca ctt atc 1695
Thr Ile Leu Thr Ser Cys Tyr Lys Tyr Ser Gln Pro Asp Thr Leu Ile
440 445 450

ttt gat tat agt agc att gat gaa ttt gga aat gct ttg gac agt aat 1743
Phe Asp Tyr Ser Ser Ile Asp Glu Phe Gly Asn Ala Leu Asp Ser Asn
455 460 465

tat ggg cat gga agt att tat cgt caa aaa gat ttg tgt aca agt gag 1791
Tyr Gly His Gly Ser Ile Tyr Arg Gln Lys Asp Leu Cys Thr Ser Glu

470 475 480

caa ata tta act gca ttg tct aaa gat gag ata cca aca act gca tgg 1839
Gln Ile Leu Thr Ala Leu Ser Lys Asp Glu Ile Pro Thr Thr Ala Trp
485 490 495 500

tca ttt gta aca aaa cgc tct gtg att gaa aaa cac gat tta cta ttt 1887
Ser Phe Val Thr Lys Arg Ser Val Ile Glu Lys His Asp Leu Leu Phe
505 510 515

tct gtt gga aaa aaa ttt gaa gat aac aat ttt acg ccg aaa gtt ttt 1935
Ser Val Gly Lys Lys Phe Glu Asp Asn Asn Phe Thr Pro Lys Val Phe
520 525 530

tac ttt agt aaa aac att gtt gtt att tcc cta aga ttg tat aga tat 1983
Tyr Phe Ser Lys Asn Ile Val Val Ile Ser Leu Arg Leu Tyr Arg Tyr
535 540 545

agg aaa cgc tct ggg tct att atg agt aat cgc ccg gaa aaa ttc ttt 2031
Arg Lys Arg Ser Gly Ser Ile Met Ser Asn Arg Pro Glu Lys Phe Phe
550 555 560

tcg gac gac gcc att ttt gta aca tat gac tta tta gat ttt tat gat 2079
Ser Asp Asp Ala Ile Phe Val Thr Tyr Asp Leu Leu Asp Phe Tyr Asp
565 570 575 580

cag tat aaa att cgg gaa ttg gga gca gta gtt ggt aaa ata gtt atg 2127
Gln Tyr Lys Ile Arg Glu Leu Gly Ala Val Val Gly Lys Ile Val Met
585 590 595

aca aca tta gct tct ttt cca gat tcg aaa aaa ttg tat aat gaa tta 2175
Thr Thr Leu Ala Ser Phe Pro Asp Ser Lys Lys Leu Tyr Asn Glu Leu
600 605 610

aat cca atc aga aaa aaa gta ttt aaa gat tat att tca ata gaa aaa 2223
Asn Pro Ile Arg Lys Lys Val Phe Lys Asp Tyr Ile Ser Ile Glu Lys
615 620 625

aga cat act aaa cgg ata aaa atg tat gta aaa atg tat gtt ttt tct 2271
Arg His Thr Lys Arg Ile Lys Met Tyr Val Lys Met Tyr Val Phe Ser
630 635 640

tct tat gtt gga tat aaa ctt tac aga ctg gta aaa ggt aaa cac tgg 2319
Ser Tyr Val Gly Tyr Lys Leu Tyr Arg Leu Val Lys Gly Lys His Trp
645 650 655 660

aag tgaatataat ttttaatctt atttatg 2349
Lys

<210> 13
<211> 332
<212> PRT
<213> Lactoccocus Lactis

<400> 13

Leu Ser Glu Asn Leu Ile Ser Ile Ile Val Pro Val Tyr Asn Ser Glu
1 5 10 15

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Lys Tyr Leu Arg Ala Ala Ile His Ser Leu Leu Asn Gln Thr Tyr Gln
            20                  25                  30

Asn Ile Glu Val Ile Leu Ile Asn Asp Gly Ser Thr Asp Gly Ser Gln
        35                  40                  45

Glu Leu Ile Ser Ser Phe Gln Lys Lys Asp Lys Arg Ile Lys Leu Tyr
    50                  55                  60

Asn Thr Lys Asn Leu Gly Val Ser His Ala Arg Asn Tyr Gly Ile Asp
65                  70                  75                  80

Arg Ala Ser Gly Ser Tyr Ile Met Phe Leu Asp Pro Asp Asp Thr Tyr
                85                  90                  95

Asp Lys Ser Tyr Cys Leu Glu Met Ile Gly Leu Ile Asn Lys Phe Asn
            100                 105                 110

Ala Asp Val Val Met Ser Asn Tyr Tyr Ile Cys Lys Gly Lys Asn Ile
        115                 120                 125

Tyr Pro Asn Val Asn Asn Asp Leu Leu Glu Cys Glu Gly Leu Leu Ser
    130                 135                 140

Arg Asp Lys Thr Met Arg Ser Ile Leu Ser Asp Thr Gly Phe Lys Gly
145                 150                 155                 160

Phe Val Trp Thr Arg Ile Phe Arg Lys Asn Val Ile Asn Asn Val Lys
                165                 170                 175

Phe Asn Glu Ser Ile Asn Tyr Leu Glu Asp Met Leu Phe Asn Ile Ser
            180                 185                 190

Ile Val His Asn Ala Arg Ile Ile Ala Tyr Thr Asn Lys Arg His Tyr
        195                 200                 205

Phe Tyr Leu Gln Arg Glu Asp Ser Ala Ser Lys Lys Phe Ser Lys Ser
    210                 215                 220

Phe Phe Lys Ser Leu Asn Leu Ile Arg Gly Lys Val Asp Pro Glu Phe
225                 230                 235                 240

Tyr Ser Gln Ile Asp Ser Val Ile Phe Tyr Asn Leu Val Gly Trp Leu
                245                 250                 255

Ile Thr Glu Arg Lys Ser Arg Glu Asn Ser Gln Phe Ile Arg Arg Asn
            260                 265                 270

Ile Lys Asn Met Lys Ser Gln Val Lys Phe Lys Thr Leu Lys Met Glu
        275                 280                 285

Asn Pro Ile Lys Asn Leu Ile Leu Lys Leu Ser Tyr Ala Phe Pro Leu
    290                 295                 300

Val Gly Ser Cys Met Ile His Met Leu Ser Val Phe Met Lys Thr Lys
305                 310                 315                 320

Leu Tyr Ser Lys Leu Met Ser Met Leu Arg Lys Gly
                325                 330
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<210> 14
<211> 329
<212> PRT
<213> Lactococcus Lactis

<400> 14

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Met Asn Pro Leu Ile Ser Ile Ile Val Pro Ile Tyr Asn Val Glu Lys
1               5                   10                  15

Tyr Ile Gly Ser Leu Val Asn Ser Leu Leu Lys Gln Thr Asn Lys Asn
            20                  25                  30

Phe Glu Val Ile Phe Ile Asp Asp Gly Ser Thr Asp Glu Ser Met Gln
        35                  40                  45

Ile Leu Lys Glu Ile Met Ala Gly Ser Glu Gln Glu Phe Ser Phe Lys
    50                  55                  60

Leu Leu Gln Gln Val Asn Gln Gly Leu Ser Ser Ala Arg Asn Ile Gly
65                  70                  75                  80

Ile Leu Asn Ala Thr Gly Glu Tyr Ile Phe Phe Leu Asp Ser Asp Asp
                85                  90                  95

Glu Ile Glu Ser Asn Phe Val Glu Thr Ile Leu Thr Ser Cys Tyr Lys
            100                 105                 110

Tyr Ser Gln Pro Asp Thr Leu Ile Phe Asp Tyr Ser Ser Ile Asp Glu
        115                 120                 125

Phe Gly Asn Ala Leu Asp Ser Asn Tyr Gly His Gly Ser Ile Tyr Arg
    130                 135                 140

Gln Lys Asp Leu Cys Thr Ser Glu Gln Ile Leu Thr Ala Leu Ser Lys
145                 150                 155                 160

Asp Glu Ile Pro Thr Thr Ala Trp Ser Phe Val Thr Lys Arg Ser Val
                165                 170                 175

Ile Glu Lys His Asp Leu Leu Phe Ser Val Gly Lys Lys Phe Glu Asp
            180                 185                 190

Asn Asn Phe Thr Pro Lys Val Phe Tyr Phe Ser Lys Asn Ile Val Val
        195                 200                 205

Ile Ser Leu Arg Leu Tyr Arg Tyr Arg Lys Arg Ser Gly Ser Ile Met
    210                 215                 220

Ser Asn Arg Pro Glu Lys Phe Phe Ser Asp Asp Ala Ile Phe Val Thr
225                 230                 235                 240

Tyr Asp Leu Leu Asp Phe Tyr Asp Gln Tyr Lys Ile Arg Glu Leu Gly
                245                 250                 255

Ala Val Val Gly Lys Ile Val Met Thr Thr Leu Ala Ser Phe Pro Asp
            260                 265                 270

Ser Lys Lys Leu Tyr Asn Glu Leu Asn Pro Ile Arg Lys Lys Val Phe
        275                 280                 285
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Lys Asp Tyr Ile Ser Ile Glu Lys Arg His Thr Lys Arg Ile Lys Met
    290                 295                 300

Tyr Val Lys Met Tyr Val Phe Ser Ser Tyr Val Gly Tyr Lys Leu Tyr
305                 310                 315                 320

Arg Leu Val Lys Gly Lys His Trp Lys
                325


<210> 15
<211> 1612
<212> DNA
<213> Lactococcus lactis

<220>
<221> CDS
<222> (76)..(1488)

<400> 15
ggtggacagg aggacacaat ttttaatcct tcctgttata tagtttttgt ttaatatttt 60

tcgggagggt tatta atg caa atc gca aaa aat tat ctt tat aat gca ata  111
                 Met Gln Ile Ala Lys Asn Tyr Leu Tyr Asn Ala Ile
                 1               5                   10

tat cag gtc ttt ata ata att gtg cca tta ctt acc att cct tat ttg   159
Tyr Gln Val Phe Ile Ile Ile Val Pro Leu Leu Thr Ile Pro Tyr Leu
        15                  20                  25

tca aga att ttg ggc cct tca ggt att gga att aac tca tat acc aat   207
Ser Arg Ile Leu Gly Pro Ser Gly Ile Gly Ile Asn Ser Tyr Thr Asn
    30                  35                  40

tct att gtt cag tat ttt gtt tta ttt ggt agt ata gga gtc ggt ttg   255
Ser Ile Val Gln Tyr Phe Val Leu Phe Gly Ser Ile Gly Val Gly Leu
45                  50                  55                  60

tat ggg aat cgt cag att gcc ttt gtt agg gat aat cag gtc aaa atg   303
Tyr Gly Asn Arg Gln Ile Ala Phe Val Arg Asp Asn Gln Val Lys Met
                65                  70                  75

tct aaa gtc ttt tat gaa ata ttt att tta aga cta ttt aca ata tgt   351
Ser Lys Val Phe Tyr Glu Ile Phe Ile Leu Arg Leu Phe Thr Ile Cys
            80                  85                  90

tta gca tat ttt ttg ttc gtt gct ttt tta atc att aat ggt cag tat   399
Leu Ala Tyr Phe Leu Phe Val Ala Phe Leu Ile Ile Asn Gly Gln Tyr
        95                  100                 105

cat gca tac tat ttg tct caa tcc att gct ata gtt gca gct gca ttt   447
His Ala Tyr Tyr Leu Ser Gln Ser Ile Ala Ile Val Ala Ala Ala Phe
    110                 115                 120

gat atc tct tgg ttt ttt atg gga att gaa aat ttt aaa gta act gta   495
Asp Ile Ser Trp Phe Phe Met Gly Ile Glu Asn Phe Lys Val Thr Val
125                 130                 135                 140

tta aga aat ttt ata gtt aag tta ctt gct cta ttc agt att ttc cta   543
Leu Arg Asn Phe Ile Val Lys Leu Leu Ala Leu Phe Ser Ile Phe Leu
                145                 150                 155
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ttt gtc aaa tct tac aat gat ttg aat ata tat ata ttg ata aca gtt    591
Phe Val Lys Ser Tyr Asn Asp Leu Asn Ile Tyr Ile Leu Ile Thr Val
            160                 165                 170

tta tct aca tta att ggt aat tta act ttt ttc cca agt tta cac aga    639
Leu Ser Thr Leu Ile Gly Asn Leu Thr Phe Phe Pro Ser Leu His Arg
        175                 180                 185

tat ctc gta aag gtt aac tat cgt gaa tta agg cca ata aag cat tta    687
Tyr Leu Val Lys Val Asn Tyr Arg Glu Leu Arg Pro Ile Lys His Leu
    190                 195                 200

aag caa tct tta gtc atg ttt atc cca caa att gct gtc caa att tat    735
Lys Gln Ser Leu Val Met Phe Ile Pro Gln Ile Ala Val Gln Ile Tyr
205                 210                 215                 220

tgg gtt ttg aat aaa acg atg tta ggt tca ttg gat tct gtc acg agc    783
Trp Val Leu Asn Lys Thr Met Leu Gly Ser Leu Asp Ser Val Thr Ser
                225                 230                 235

tcc ggc ttt ttt gat cag tct gat aaa ata gtt aaa ctg gtt ttg gct    831
Ser Gly Phe Phe Asp Gln Ser Asp Lys Ile Val Lys Leu Val Leu Ala
            240                 245                 250

att gct act gca aca ggt act gtc atg ttg cca cgt gtt gca aat gcc    879
Ile Ala Thr Ala Thr Gly Thr Val Met Leu Pro Arg Val Ala Asn Ala
        255                 260                 265

ttt gca cat aga gag tat agt aaa att aag gaa tac atg tac gca ggt    927
Phe Ala His Arg Glu Tyr Ser Lys Ile Lys Glu Tyr Met Tyr Ala Gly
    270                 275                 280

ttt tct ttt gtg tcg gca att tcg att cct atg atg ttt ggt ctg ata    975
Phe Ser Phe Val Ser Ala Ile Ser Ile Pro Met Met Phe Gly Leu Ile
285                 290                 295                 300

gct att act cct aaa ttc gtg cca ctt ttt ttt aca tct caa ttt agt    1023
Ala Ile Thr Pro Lys Phe Val Pro Leu Phe Phe Thr Ser Gln Phe Ser
                305                 310                 315

gat gtt att cct gtg tta atg atc gag tca atc gca att att ttt ata    1071
Asp Val Ile Pro Val Leu Met Ile Glu Ser Ile Ala Ile Ile Phe Ile
            320                 325                 330

gct tgg agc aac gca ata ggt act caa tat ctt tta cca act aat caa    1119
Ala Trp Ser Asn Ala Ile Gly Thr Gln Tyr Leu Leu Pro Thr Asn Gln
        335                 340                 345

aat aag tca tat aca gtg tcg gtg atc att gga gcg ata gtc aat tta    1167
Asn Lys Ser Tyr Thr Val Ser Val Ile Ile Gly Ala Ile Val Asn Leu
    350                 355                 360

atg tta aat att cca ctg att ata tat cta ggt act gtt ggt gca tca    1215
Met Leu Asn Ile Pro Leu Ile Ile Tyr Leu Gly Thr Val Gly Ala Ser
365                 370                 375                 380

att gca act gta att tct gaa atg tct gta act gtg tat caa ctt ttt    1263
Ile Ala Thr Val Ile Ser Glu Met Ser Val Thr Val Tyr Gln Leu Phe
                385                 390                 395
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ata att cat aaa cag ctt aat ttg cat aca ctg ttt gcg gat tta tct   1311
Ile Ile His Lys Gln Leu Asn Leu His Thr Leu Phe Ala Asp Leu Ser
            400                 405                 410

aag tat tta att gca gga tta gtg atg ttt cta att gtc ttt aaa att   1359
Lys Tyr Leu Ile Ala Gly Leu Val Met Phe Leu Ile Val Phe Lys Ile
        415                 420                 425

agt ttg tta aca ccg aca tct tgg ata ttc att ctg ttg gaa att act   1407
Ser Leu Leu Thr Pro Thr Ser Trp Ile Phe Ile Leu Leu Glu Ile Thr
    430                 435                 440

gtg ggc ata att att tat gtt gtt tta tta ata ttt tta aag gca gaa   1455
Val Gly Ile Ile Ile Tyr Val Val Leu Leu Ile Phe Leu Lys Ala Glu
445                 450                 455                 460

ata att aat aag cta aag ttt att atg cat aaa tagaggtatg gatttaggta 1508
Ile Ile Asn Lys Leu Lys Phe Ile Met His Lys
                465                 470

cctgccttat tgaaaataac ggtgagtcaa tggtattggg catatttgac gctcaccttc 1568

aatttgtttt ggtcgacttg attgtagcac aggacaatat gtct                  1612


&lt;210&gt; 16
&lt;211&gt; 471
&lt;212&gt; PRT
&lt;213&gt; Lactococcus lactis

&lt;400&gt; 16
Met Gln Ile Ala Lys Asn Tyr Leu Tyr Asn Ala Ile Tyr Gln Val Phe
1               5                   10                  15

Ile Ile Ile Val Pro Leu Leu Thr Ile Pro Tyr Leu Ser Arg Ile Leu
            20                  25                  30

Gly Pro Ser Gly Ile Gly Ile Asn Ser Tyr Thr Asn Ser Ile Val Gln
        35                  40                  45

Tyr Phe Val Leu Phe Gly Ser Ile Gly Val Gly Leu Tyr Gly Asn Arg
    50                  55                  60

Gln Ile Ala Phe Val Arg Asp Asn Gln Val Lys Met Ser Lys Val Phe
65                  70                  75                  80

Tyr Glu Ile Phe Ile Leu Arg Leu Phe Thr Ile Cys Leu Ala Tyr Phe
                85                  90                  95

Leu Phe Val Ala Phe Leu Ile Ile Asn Gly Gln Tyr His Ala Tyr Tyr
            100                 105                 110

Leu Ser Gln Ser Ile Ala Ile Val Ala Ala Ala Phe Asp Ile Ser Trp
        115                 120                 125

Phe Phe Met Gly Ile Glu Asn Phe Lys Val Thr Val Leu Arg Asn Phe
    130                 135                 140

Ile Val Lys Leu Leu Ala Leu Phe Ser Ile Phe Leu Phe Val Lys Ser
145                 150                 155                 160
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Tyr Asn Asp Leu Asn Ile Tyr Ile Leu Ile Thr Val Leu Ser Thr Leu
                165                 170                 175

Ile Gly Asn Leu Thr Phe Phe Pro Ser Leu His Arg Tyr Leu Val Lys
            180                 185                 190

Val Asn Tyr Arg Glu Leu Arg Pro Ile Lys His Leu Lys Gln Ser Leu
        195                 200                 205

Val Met Phe Ile Pro Gln Ile Ala Val Gln Ile Tyr Trp Val Leu Asn
    210                 215                 220

Lys Thr Met Leu Gly Ser Leu Asp Ser Val Thr Ser Ser Gly Phe Phe
225                 230                 235                 240

Asp Gln Ser Asp Lys Ile Val Lys Leu Val Leu Ala Ile Ala Thr Ala
                245                 250                 255

Thr Gly Thr Val Met Leu Pro Arg Val Ala Asn Ala Phe Ala His Arg
            260                 265                 270

Glu Tyr Ser Lys Ile Lys Glu Tyr Met Tyr Ala Gly Phe Ser Phe Val
        275                 280                 285

Ser Ala Ile Ser Ile Pro Met Met Phe Gly Leu Ile Ala Ile Thr Pro
    290                 295                 300

Lys Phe Val Pro Leu Phe Phe Thr Ser Gln Phe Ser Asp Val Ile Pro
305                 310                 315                 320

Val Leu Met Ile Glu Ser Ile Ala Ile Ile Phe Ile Ala Trp Ser Asn
                325                 330                 335

Ala Ile Gly Thr Gln Tyr Leu Leu Pro Thr Asn Gln Asn Lys Ser Tyr
            340                 345                 350

Thr Val Ser Val Ile Ile Gly Ala Ile Val Asn Leu Met Leu Asn Ile
        355                 360                 365

Pro Leu Ile Ile Tyr Leu Gly Thr Val Gly Ala Ser Ile Ala Thr Val
    370                 375                 380

Ile Ser Glu Met Ser Val Thr Val Tyr Gln Leu Phe Ile Ile His Lys
385                 390                 395                 400

Gln Leu Asn Leu His Thr Leu Phe Ala Asp Leu Ser Lys Tyr Leu Ile
                405                 410                 415

Ala Gly Leu Val Met Phe Leu Ile Val Phe Lys Ile Ser Leu Leu Thr
            420                 425                 430

Pro Thr Ser Trp Ile Phe Ile Leu Leu Glu Ile Thr Val Gly Ile Ile
        435                 440                 445

Ile Tyr Val Val Leu Leu Ile Phe Leu Lys Ala Glu Ile Ile Asn Lys
    450                 455                 460

Leu Lys Phe Ile Met His Lys
465                 470
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